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Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

ISSN 0706-652X / CJFSDX 53(5) 943–1202 (1996)

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Volume 53, Number 5, May 1996

Canadian Journal of Fisheries and Aquatic Sciences

Published by the National Research Council of Canada

Volume 53, numéro 5, mai 1996

Journal canadien des sciences halieutiques et aquatiques

Publié par le Conseil national de recherches du Canada

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Spatial and temporal variation in the density of northern cod and a review of hypotheses for the stock's collapse[1]

Jeffrey A. Hutchings

Abstract: The collapse of northern cod, *Gadus morhua*, off Newfoundland and Labrador was associated with clearly defined spatial and temporal changes in density and biomass. Between 1981 and 1992, low density research survey tows (<100 kg/tow) increased gradually from 76 to 97% concomitant with a gradual decline in medium density tows (100–500 kg/tow) from 22 to 2%. By contrast, high density tows (>500 kg/tow) remained proportionately constant (~1.5%) until 1992, whereafter they declined to zero. Southward, spatio-temporal changes in stock biomass were unaccompanied by a shift in cod distribution. A simple density composition model provides a biological basis for observed changes in mobile and fixed-gear catch rates, increased catchability of cod with declining stock biomass, and rapid increases in fishing mortality. A nested aggregation model of a small, constant number of dense cod aggregations, each encompassed by, and recruited from, lower density areas, explains how cod vulnerability to fishing can increase with declining stock biomass. A review of recent research identifies excessive fishing mortality as the sole significant cause of northern cod's collapse. Prevention of fishery collapses arguably rests on the dominant question to emerge from this review: what are the effects of fishing on the behaviour, life history, and population biology of exploited fishes?

Résumé : L'effondrement des stocks de morue, *Gadus morhua*, au large de Terre-Neuve et du Labrador est associé à des changements spatio-temporels nets de densité et de biomasse. Entre 1981 et 1992, les traits de dénombrement à faible densité de capture (<100 kg par trait) se sont faits de plus en plus fréquents, passant de 76 à 97%, tandis que les traits à densité moyenne (100–500 kg par trait) sont passés de 22 à 2%. Toutefois, les traits à forte densité de capture (>500 kg par trait) sont restés sensiblement constants (≈1,5%) jusqu'en 1992 avant de tomber à zéro. Vers le sud, les changements spatio-temporels de la biomasse des stocks ne se sont pas accompagnés d'une modification de la répartition de la morue. Un modèle simple de composition par densité offre un fondement biologique aux changements observés relativement aux taux de capture au moyen d'engins mobiles et d'engins fixes, à la capturabilité accrue de la morue à mesure que la biomasse des stocks diminue, et à la mortalité par pêche, qui est en hausse rapide. Un modèle d'agrégation à plusieurs degrés décrivant un petit nombre constant de denses concentrations de morue, chacune circonscrite par des zones de concentration moindre d'où se fait le recrutement dans les concentrations élevées, permet d'expliquer comment la vulnérabilité à la pêche de la morue peut s'intensifier à mesure que la biomasse du stock diminue. Une recherche documentaire montre que la très forte mortalité par pêche est l'unique cause importante de l'effondrement des stocks de morue. Il est logique de penser que pour éviter l'effondrement des pêches, on doit s'attaquer à la question centrale que cette recherche a fait apparaître : quels sont les effets de la pêche sur le comportement, le cycle biologique et la biologie des populations exploitées?
[Traduit par la Rédaction]

Introduction

The collapse of the Atlantic cod, *Gadus morhua*, fisheries off eastern Canada has prompted considerable debate concerning the question of whether excessive fishing pressure or excessive natural mortality was the primary cause of the stock declines (e.g., deYoung and Rose 1993; Lear and Parsons 1993; Atkinson and Bennett 1994; Hutchings and Myers 1994*a*, 1995; Myers and Cadigan 1995*a*, 1995*b*; Myers et al. 1996*a*, 1996*b*). Such debate is healthy and should serve to narrow the range of potential causes to the point that new research initiatives and management strategies that reduce the likelihood of recurrence of these collapses will be identified and implemented.

The northern cod stock, once the world's largest (McGrath 1911), experienced a collapse considerably more severe than that of other Canadian east coast cod stocks. Between 1962 (the first year for which age-specific abundance estimates are available) and 1992, the estimated spawner biomass of northern cod had declined almost two orders of magnitude (by contrast, the mean size of the spawner biomass of other cod stocks at the time of their collapse was 13% of their historical maxima; Myers et al. 1996*b*). The concomitant 94% decrease in recruitment (the abundance of cod aged 3 years, the age at which cod are first vulnerable to commercial fishing gear) is not surprising given the strong stock-recruitment relationship that appears to exist for this stock (Hutchings and Myers 1994*a*). Northern cod spawn throughout the continental shelf from Hamilton Bank south to Grand Bank (Hutchings et al. 1993) with approximately 50% of males and females maturing at 5 and 6 years, respectively (Taggart et al. 1994). Tagging

Received March 13, 1996. Accepted May 24, 1996.
J13350

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[1] J.C. Stevenson Memorial Lecture, delivered 5 January 1996, Montréal, Quebec.

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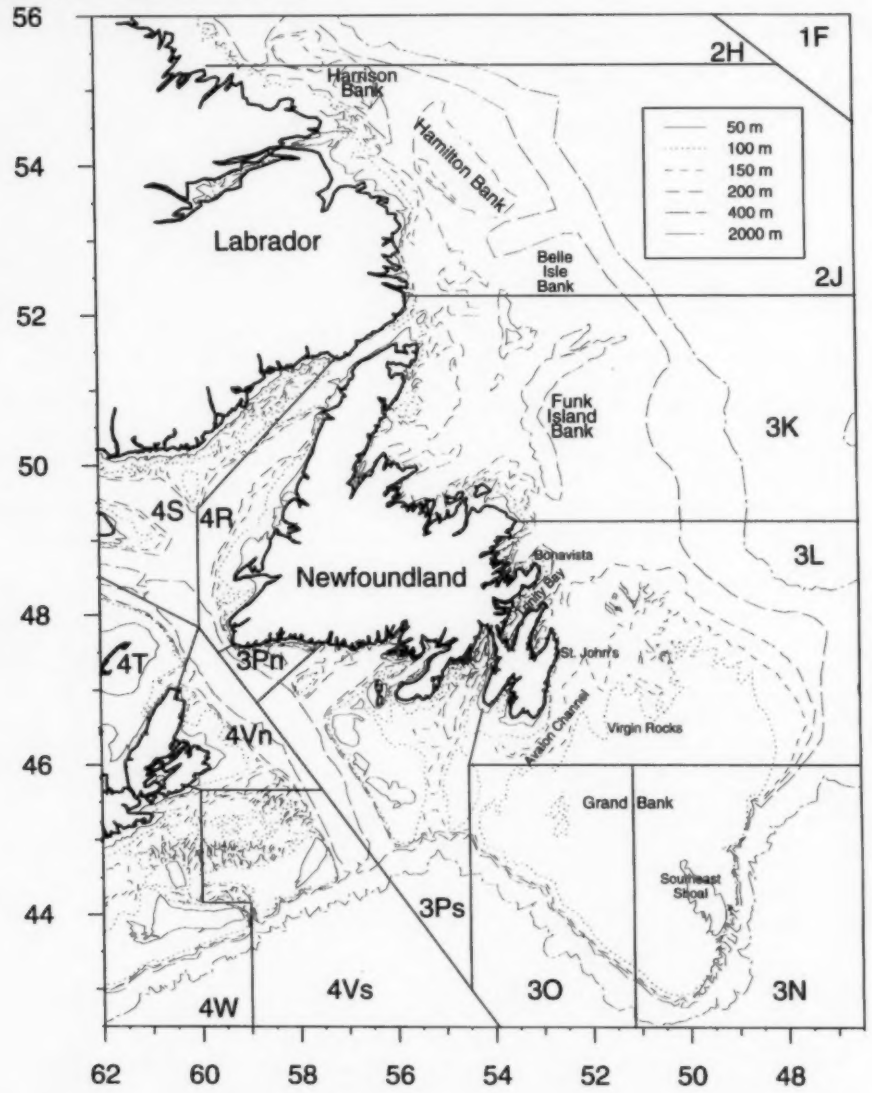
Fig. 1. Map showing the Northwest Atlantic Fishery Organisation (NAFO) management units off Newfoundland and Labrador. The northern cod stock is delineated by NAFO divisions 2J, 3K, and 3L.



studies (Templeman 1965; Lear 1984) indicate that northern cod undergo spring feeding migrations from offshore regions to inshore waters, where they disperse among the headlands and bays and become vulnerable to fixed fishing gear (e.g., gill nets, cod traps, line trawls, hand lines). These cod appear to move offshore again in autumn and remain there during winter when they form prespawning and spawning aggregations that render them highly vulnerable to the mobile fishing fleets that target these aggregations (Hutchings 1995; Kulka et al. 1995). Although the stock is managed as a single unit encompassing Northwest Atlantic Fishery Organisation, or NAFO, divisions 2J, 3K, and 3L (Fig. 1), there is considerable evidence of separate inshore and offshore stocks based on analyses of tagging experiments (Templeman 1965; Lear 1984; Myers et al. 1997), genetic data (Ruzzante et al. 1996), spawning locations (Hutchings et al. 1993), and the local knowledge of fishermen (Hutchings et al. 1996; Neis et al.[2]).

Debate concerning the causes of the decline of northern cod has occurred largely in the absence of a spatio-temporal model of the density distribution of cod. An exception is the work by Warren (1997) who, on the basis of an analysis that separated the autocorrelation in the research survey data into its spatial and temporal components, concluded that the spatial structure

[2] B. Neis, J.A. Hutchings, R.L Haedrich, D. Schneider, and L. Felt. Scientific debates, disappearing lumpfish and sticky nets: fishers' ecological knowledge and adaptive management. Submitted for publication.

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of northern cod had changed from a highly structured stock in 1985 to one possessing little structure in 1992. It has been hypothesized that cod shifted their distribution southward just prior to the stock collapse (deYoung and Rose 1993; Rose et al. 1994) and that cod became increasingly concentrated through the late 1980s and early 1990s (Bishop et al. 1993). If true, such changes in distribution and density composition may have increased the vulnerability of cod to fishing gear and accelerated the stock's rate of decline.

My objectives are to examine research survey data on northern cod to describe spatial and temporal variability in cod density, to demonstrate how such information can be used to assess the relative importance of fishing and the environment to the stock's collapse, and to illustrate how such data can be used to identify criteria upon which the re-opening of collapsed fisheries might be based. I construct simple models to describe the numerical changes in cod density (density composition model) and the temporal changes in the spatial structure of cod (nested aggregation model) that were concurrent with declining abundance of northern cod through the 1980s and early 1990s. To place these models, and their predictions, into a broader context, I review recent research on the causes of the collapse of northern cod. Lastly, I focus upon research initiatives that may serve to increase our understanding of the effects of fishing on exploited populations and identify means of ensuring their long-term persistence.

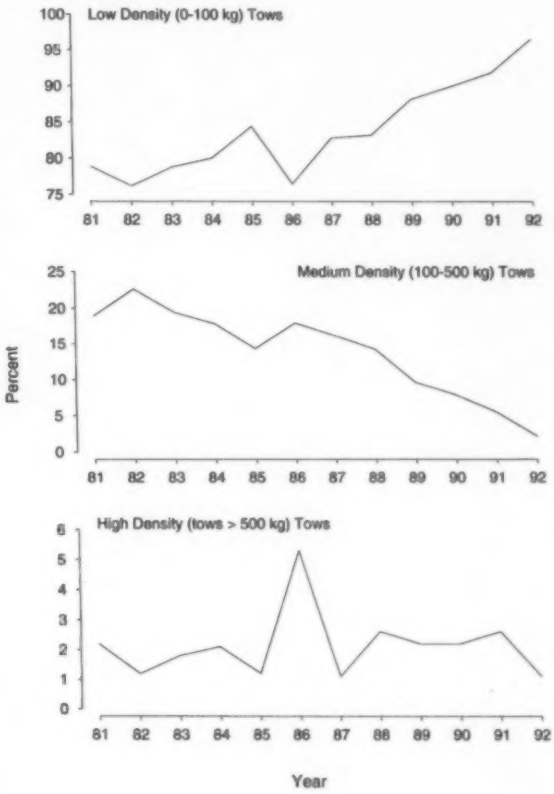
Density composition of northern cod

Research surveys have sampled the entire 2J3KL area over a relatively short period of time (8–10 weeks in most years) every autumn (October through December) since 1981. The survey area is divided into 76 sections called strata, the boundaries of which are delineated primarily by depth. Each stratum is sampled every year, although the within-stratum locations of each half-hour, approx. 0.046-km^2 tow are chosen at random (2–26 tows per stratum between 1981 and 1992). With the exception of the relatively early 1984 3L survey, the timing of the surveys has not changed significantly through time. Given that the annual timing of the peak inshore catches did not change significantly from 1981 to 1990 ($F_{[1,7]} = 1.55$; $p = 0.253$), it would appear that the timing of the autumnal offshore migration by cod changed little over the same period.

I grouped research survey tows conducted between 1981 and 1992 into low (0–100 kg), medium (100–500 kg), and high biomass (greater than 500 kg) tows. Given that each tow samples approximately the same swept area, these groups can be identified as low, medium, and high density tows. These would correspond to 0 to 2.2, 2.2 to 11.0, and greater than 11.0 t/km^2 (to a maximum of 139 t/km^2 in 1986), respectively. The percentage of tows in each density class was calculated for the entire area sampled by the autumn surveys in 2J3KL for each year. Tows in which the trawling gear did not operate properly were excluded from the analysis.

The percentages of tows by density class showed consistent trends over time (Fig. 2). Of all tows conducted annually from 1981 to 1984, roughly 78% were low density tows, 20% were medium density tows, and 2% sampled cod at high densities. Omitting the 1986 datum when sampling conditions resulted in unusually high catchabilities of cod, low density tows in the mid–1980s (1985 to 1988) increased in prevalence and medium density tows decreased while the percentage of high density tows remained largely unchanged. The annual percentage of high density tows remained constant at roughly 2% through 1991 while the percentages of low density tows had increased to 92% and medium density tows had declined to 6%. By 1992, low density tows constituted 97% of all tows with medium and high density tows constituting approximately 2% and 1% of all tows, respectively. There have been no high density research tows of northern cod since 1992 (Department of Fisheries and Oceans, unpublished data).

Fig. 2. Temporal changes in the proportional representation of low (<100 kg), medium (100–500 kg), and high (>500 kg) biomass tows in the autumn research survey data for northern cod from 1981 to 1992.


Low Density (0-100 kg) Tows
Medium Density (100-500 kg) Tows
High Density (tows > 500 kg) Tows
Percent
Year
81 82 83 84 85 86 87 88 89 90 91 92

Density composition model

The temporal pattern in density composition of tows may reflect predictable responses by cod to declining stock biomass. I constructed simple models to describe observed changes in the proportions of low, medium, and high density tows of cod over time:

(1*a*) $\alpha_{L(t)} = \alpha_{L(t=0)} + \beta_{L1}(t)$, when $\alpha_M > \alpha_{M^*}$

(1*b*) $\alpha_{L(t)} = \alpha_{L(t=t^*)} + \beta_{L2}(t-t^*)$, when $\alpha_M \leq \alpha_{M^*}$

(2*a*) $\alpha_{M(t)} = \alpha_{M(t=0)} - \beta_{M1}(t)$, when $\alpha_M > \alpha_{M^*}$

(2*b*) $\alpha_{M(t)} = \alpha_{M(t=t^*)} - \beta_{M2}(t-t^*)$, when $\alpha_M \leq \alpha_{M^*}$

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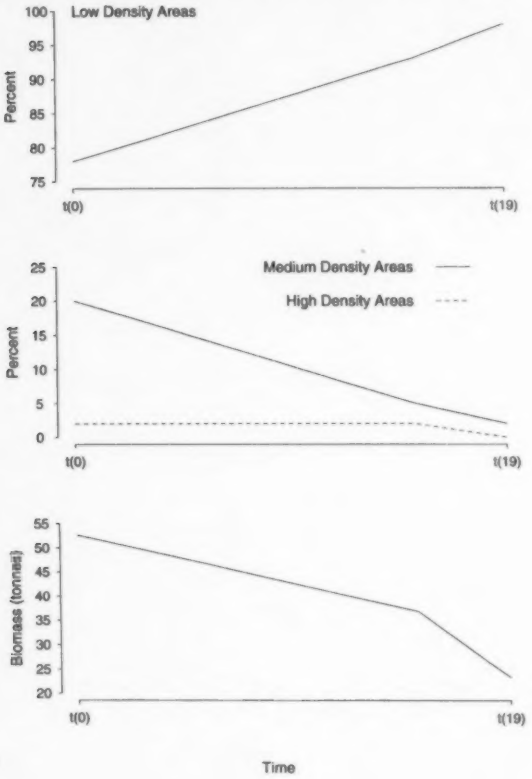
Fig. 3. Temporal changes in the proportional representation of low, medium, and high density areas of northern cod corresponding to tthe density composition model described by eqs. 1–3 in the text. Changes in total stock biomass associated with this model (cf. eq. 4) are presented in the bottom panel. Time is in arbitrary units.



$$(3a) \quad \alpha_{H(t)} = \alpha_{H(t=0)}, \text{ when } \alpha_M > \alpha_{M^*}$$

$$(3b) \quad \alpha_{H(t)} = \alpha_{H(t=t^*)} - \beta_H(t-t^*), \text{ when } \alpha_M \leq \alpha_{M^*}$$

From initial values at time $t = 0$ (i.e., $\alpha_{L(t=0)}$ and $\alpha_{M(t=0)}$), the proportion of low and medium density tows increases and decreases, respectively, at a constant rate ($\beta_{L1} = \beta_{M1} = 0.01\ t^{-1}$) until time = t^*, when the percentage of medium tows reaches a threshold, whereafter the rates of change decline ($\beta_{L2} = 0.0075\ t^{-1}$; $\beta_{M2} = 0.0050\ t^{-1}$). The proportion of high density tows is modelled to remain constant at its initial value, $\alpha_{H(t=0)}$, until t^*, whereafter the proportion of high density tows declines at a constant rate of $\beta_H = 0.005\ t^{-1}$. (Values of β for each density class were chosen to be consistent with empirical data; Fig. 2.)

Concomitant with changes in density composition are changes in stock biomass, $N_{(t)}$, where

$$(4) \quad N_{(t)} = \mu_L T\alpha_{L(t)} + \mu_M T\alpha_{M(t)} + \mu_H T\alpha_{H(t)}$$

The total number of tows per year, T, equalled the 1981–1992 average of 424. The mean biomasses of cod in low, medium, and high density tows were $\mu_L = 50$ kg, $\mu_M = 300$ kg, and $\mu_H = 1260$ kg, respectively. These values represent the mid-range biomass values for the low and medium density tows and the 1981–1992 average of high density tows.

The temporal trends described by the density composition model (eqs. 1–3; Fig. 3) are similar to, albeit less variable than, those observed in the research survey data (Fig. 2). Changes in model stock biomass with changes in density (Fig. 3, lower panel) can be used to predict temporal changes in biomass and mortality indices often quantified in stock assessments. I examine three such indices: (*i*) catch rates of mobile and fixed gear, (*ii*) catch rate as a predictor of stock biomass, and (*iii*) fishing mortality.

As stock biomass declines, the density composition model predicts different trends in the catch rates of cod in high and in medium–low density areas. Catch rates in high density areas are predicted to remain relatively stable before declining sharply when the percentage of medium density tows falls below a threshold level (5% in the present model, as observed in the northern cod research surveys). Catch rates in medium and low density areas, however, are predicted to decrease continuously with declining stock biomass (Fig. 4A). These predicted catch rate trends in high and medium–low density areas correspond to those observed in the trawler and gill-net sectors of the northern cod fishery, respectively (Fig. 5A). (Trawler and gill-net catch rate data are expressed as proportions of their respective maxima.) Trawler catch rates changed relatively little from 1982 to 1989, declining at a rate of about 2% per year, before declining 16% per annum thereafter. In constrast, gill-net catch rates in the inshore (from 1985, the first year for which data are available) and offshore fisheries (from its initiation in 1986) declined at the considerably faster rates of 13 and 14%, respectively (data from Hutchings and Myers 1994*a*).

Until 1988, the catch rate of commercial trawlers was assumed to be proportional to the size of the northern cod stock (Baird et al. 1991). The density composition model predicts that catch rates in high density areas should remain constant, despite declining stock biomass, until stock size falls to a sufficiently low level whereafter catch rate declines proportionately with stock biomass (Fig. 4B). This predicted relationship between catch rate in high density areas and stock biomass corresponds with that observed between commercial trawler catch rate and harvestable northern cod biomass from 1982 to 1991 (Fig. 5B). Thus, the density composition model provides an explanation for the negative relationship between catchability and northern cod biomass documented by Hutchings and Myers (1994*a*).

The density composition model predicts that the instantaneous rate of fishing mortality, F, associated with the decline in stock biomass increases relatively slowly prior to a comparatively short period in which F increases rapidly (Fig. 4C). Here, fishing mortality in year t was determined from the equation $HR_{(t)} = 1 - e^{(-F(t))}$, where harvest rate (HR) in year t is

$$(5) \quad HR_{(t)} = \frac{\theta}{N_{L(t)} + N_{M(t)} + N_{H(t)}}$$

and where the value of θ, analogous to a constant total allowable catch (TAC), was such that F in year $t = 0$ equalled the value of F estimated for the northern cod fishery in 1982 ($F_{1982} = 0.44$; Bishop et al. 1994). Coincident with declining stock biomass, the density composition model predicted that

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Fig. 4. Temporal changes in metrics of cod biomass and stock health predicted by the density composition model (eqs. 1–3 in the text). (A) Predicted temporal changes in catch rate in high and medium–low density areas. (B) Predicted relationship between catch rate in high density areas and stock biomass. (C) Predicted changes in instantaneous fishing mortality with time. Time is given in arbitrary units.

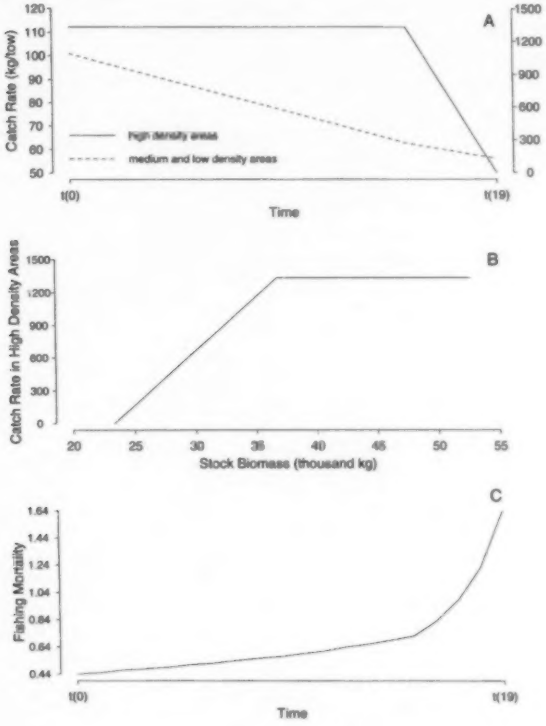
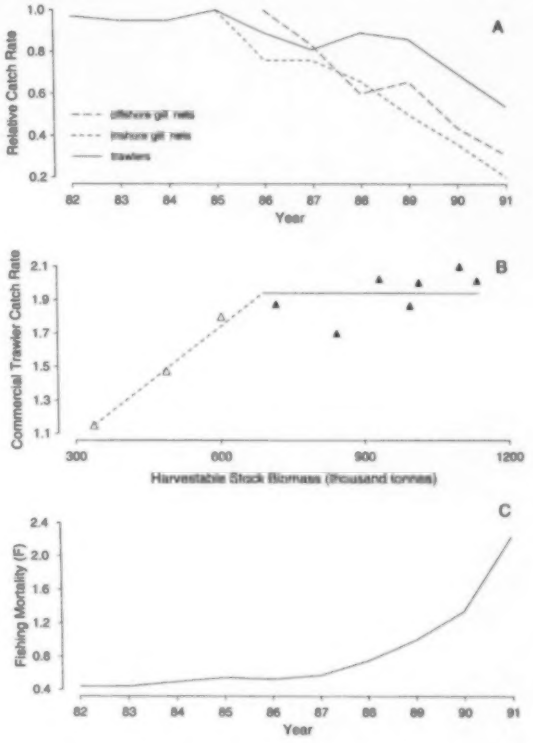


Fig. 5. Observed changes in metrics of cod biomass and stock health. (A) Temporal changes in catch rates of cod by trawlers and gill nets. (B) Relationship between trawler catch rate and harvestable biomass of northern cod (cod aged 3 years and older), as estimated by virtual population analysis (VPA). (C) Changes in instantaneous fishing mortality of northern cod, estimated by VPA, from 1982 to 1991.



F should increase slowly from 0.44 to 0.72 between $t(0)$ and $t(15)$ before rising rapidly to 1.64 by $t(19)$ (Fig. 4C). This pattern in fishing mortality predicted by the density composition model is consistent with the changes in F of northern cod fully recruited to the trawling gear (ages 7 to 9 years) estimated by virtual population analysis, or VPA (Bishop et al. 1994); between 1982 and 1988, F increased from 0.44 to 0.75 before increasing rapidly to 1.34 in 1991 and 2.23 in 1992 (Fig. 5C).

General agreement between the abundance metrics predicted by the density composition model (Fig. 4) and those observed in the northern cod fishery (Fig. 5) suggest that an understanding of the factors that contributed to the stock's collapse is incomplete in the absence of a model of the spatial structure of the northern cod stock.

How should trends in abundance and biomass be estimated from research surveys?

The similarity between predicted and observed patterns of stock abundance metrics (Figs. 4, 5), coupled with observed changes in the density composition of research tows between 1981 and 1992 (Fig. 2), indicates that northern cod were increasingly at risk of collapse from the mid-1980s. If true, why did the research survey trend in stock biomass fail to detect a decline in stock biomass until 1991 (Fig. 6A)? Either northern cod did not, in fact, begin to decline until 1991, leading to the conclusion that the stock declined rapidly between 1990 and 1991, or the means of describing trends in stock biomass was not robust to annual changes in the variance of abundance estimates and (or) changes in the stock's density composition.

Survey biomass, sometimes referred to as minimum trawlable biomass, for the 76 strata sampled during autumn in year t, $N_{(t)}$, is estimated as

$$(6) \qquad N_{(t)} = \sum_{i=1}^{76} \mu_{i(t)} \omega_i$$

where

$$\omega_i = a_i / \sum_{i=1}^{76} a_i$$

Total biomass equals the sum of the mean biomass estimates for individual strata i, μ_i, each of which is weighted by its stratum area, a_i. Annual frequency distributions of biomass per tow resemble extended Poisson distributions (i.e., long positive

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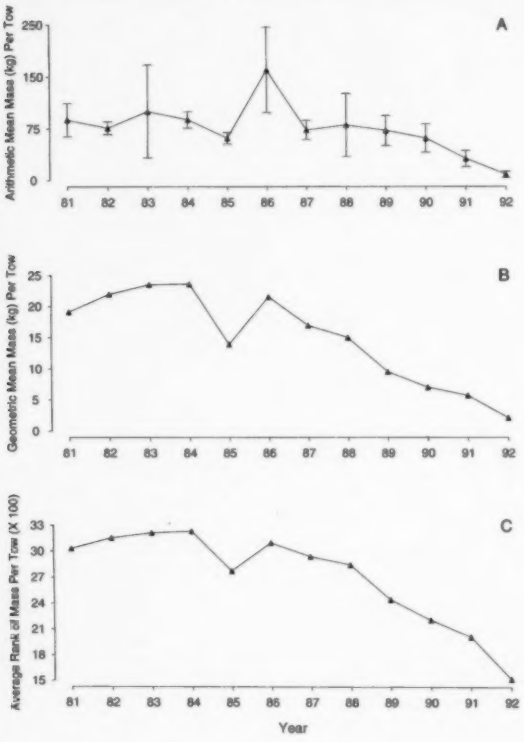
Fig. 6. Changes in (A) arithmetic mean (±SE; estimated from Bishop et al. (1994)) mass per tow, (B) geometric mean mass per tow, and (C) average rank of mass per tow of northern cod calculated from research surveys between 1981 and 1992.



skew; J.A. Hutchings, unpublished data). The justification for calculating the arithmetic mean is that it provides an unbiased estimate of biomass. However, there is concern that μ_i may not possess zero design bias, i.e., the average over all possible samples may not be the population average because of the way in which samples are taken within strata (Noel Cadigan, Department of Fisheries and Oceans, P.O. Box 5667, St. John's, NF A1C 5X1, personal communication). In addition, the standard errors of biomass estimates, often as high as 30–40%, vary considerably among years (Bishop et al. 1994).

Thus, temporal trends in stock survey biomass have been described by an estimator presumed to have no bias but high, and annually changing, variance (Fig. 6A). However, it is not clear that the criterion of zero bias necessarily warrants the use of the arithmetic mean as an estimator of abundance to the exclusion of other estimators. For example, the criterion that an abundance estimator minimize the mean squared error (MSE = bias^2 + variance) may favour the use of estimators other than the mean (Cochran 1977). A biased estimator with small variance can have a smaller MSE than an unbiased estimator with large variance.

The goal of minimizing MSE, or some other criterion that reduces variance, would seem to be justified when the objective is to examine trends in abundance as opposed to providing abundance estimates on a yearly basis. This is an important distinction. Plotting annual mean biomass estimates and using such a plot to detect abundance changes through time would only be justified if the myriad factors that influence cod abundance and their availability to the survey gear do not vary from year to year. Yearly changes in catchability of northern cod are well illustrated by the extremely high, and biologically unreasonable, 1986 biomass estimate. The assumption of constant interannual catchability has also been questioned for the southern Gulf of St. Lawrence groundfish research surveys (Swain et al. 1994).

Changes in density composition also contributed to interannual differences in the variance in biomass estimates. The proportional decline in medium density tows coupled with a stable representation of high density tows (the mean annual biomass of high density tows did not change with time; $F_{[11,109]} = 0.75$, $p = 0.69$) resulted in the mean being increasingly dependent on proportionately fewer tows. Annually, from 1981 to 1985, one half of the estimated survey biomass could be attributed to the highest 10% of the tows; by the late 1980s, 50% of the survey biomass was attributable to fewer than 2% of the tows (J.A. Hutchings, unpublished data). For example, the stock size estimated from the 1989 research surveys would have been reduced by half if it were not for 8 of the 450 tows conducted that autumn (the highest of which was sampled on the very last tow of that year's survey).

The temporal trend in arithmetic mean weight per tow suggests that northern cod biomass changed little, perhaps declining slightly, from 1981 to 1990 (Fig. 6A). The large decline between 1990 and 1991, which continued through 1992, prompted the view that the stock had declined suddenly, and unexpectedly, in 1991 (spring being the favoured time; Lear and Parsons 1993; Atkinson and Bennett 1994). However, as argued previously, interannual changes in the variance about the mean may render the plotting of annual arithmetic means to describe temporal trends problematic. To reduce the effects that a few large tows have on the mean and to reduce the effects of other sources of variability in the mean, I log-transformed the biomass per tow data (logarithmic transformations of zeroes was accommodated by adding 1 to each biomass datum), calculated the means of the log-transformed data (weighted by stratum area), and then untransformed the means to express the data in kilograms per tow (Fig. 6B).

The trend in geometric mean biomass (Fig. 6B) differs significantly from that generated by the time series of arithmetic mean biomass (Fig. 6A). The trend in geometric mean suggests that northern cod biomass peaked in 1984 and declined thereafter. This pattern of northern cod stock size is consistent with VPA-based trends in harvestable biomass (Bishop et al. 1994), catch rate data from fixed gear fisheries (Hutchings and Myers 1994*a*), and the personal experience of inshore fishermen (Neis et al., see footnote 2). Interestingly, the geometric mean significantly reduces the influence of the 1986 datum on the overall temporal trend.

A rank-based trend estimator provides another means of describing trends in stock biomass that is robust to unusually large survey catches. Here one ranks all mass per tow data for all years (the largest tow receiving the highest rank), computes an average rank of mass per tow for each year, and then plots the average ranks through time. The rank-based trend in northern cod biomass yields a pattern similar to that produced by the geometric mean (Fig. 6C); northern cod biomass peaked in

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1984 and declined thereafter. A comparison of adjacent years indicates a significant difference in rank between 1988 and 1989 (contrast test; $F_{[1,12]} = 16.97$, $p = 0.0001$) and between adjacent years thereafter (F ranging from 4.27 to 31.34, p ranging from 0.0001 to 0.0388). Contrast tests also identified a significant difference between ranks for 1984 and 1987 ($F_{[1,12]} = 9.84$, $p = 0.0017$), suggesting that 1987 was the earliest year in which a rank-based trend estimator would have detected the decline that was occurring in northern cod biomass.

The concordance between the latter trends (geometric mean and ranked mass per tow) and other indices of northern cod biomass, coupled with the lack of agreement between the trend in arithmetic means and any other metric, suggests that trends based on arithmetic means of research survey data have inaccurately reflected trends in actual stock abundance. One possible reason for this discrepancy is a violation of the assumption of randomness in survey design that resulted in an over-representation of high biomass tows in the surveys in the late 1980s (e.g., a change in the protocol for selecting alternative sampling locations because of unsuitability of original locations, increased movement of fish during the survey period). Temporal trends in biomass based on estimators other than the arithmetic mean (e.g., geometric mean, ranked mass per tow, median) may have considerable merit. One potential risk in using estimators such as the geometric mean, however, is that they may be overly sensitive to changes in fish distribution. Nonetheless, it would be prudent for stock assessments to include a comparison of biomass trends from more than one survey-based estimator of stock size. Similarity among trends would provide assurance that estimated trends reflect actual trends; disparity among trends would justify uncertainty in stock status and warrant a cautious management strategy.

Recent changes in the distribution of northern cod: real or illusory?

Previous analyses of research survey data have suggested that a dramatic change in the spatial distribution of northern cod occurred just prior to the stock collapse in 1992. The evidence for a southern shift in 1989 or 1990 has been considered strong enough that the hypothesis is now routinely accepted (e.g., Department of Fisheries and Oceans 1995; Kulka et al. 1995; Wroblewski et al. 1995). The southward shift has been thought to have been effected by a changing environment, e.g., colder water temperatures (deYoung and Rose 1993; Rose et al. 1994) and (or) ecosystem-driven shifts in prey abundance (Rose et al. 1994; Warren 1997), and is, therefore, attractive to those who assert that the collapse of northern cod was environmentally driven (see Atkinson and Bennett 1994). It has also been suggested that northern cod became increasingly concentrated (i.e., increased biomass per unit area) through the late 1980s (Bishop et al. 1993), possibly increasing the vulnerability of northern cod to commercial fishing and contributing to the rapidity of the stock decline (Rose et al. 1994). These hypotheses warrant re-examination in light of the trends in cod density revealed by the research survey data.

Spatial distribution of high and medium density aggregations of northern cod

The location of high density research tows in autumn indicates that the most dense aggregations of cod are more likely to be located near the edge of the continental shelf than they are to coastal Newfoundland and Labrador (Fig. 7). With few exceptions, high density groups are situated at depths greater than 200 m. The only area in which high concentrations of cod were consistently found at depths less than 200 m was the southern half of 3L on the northern and central portions of the Grand Bank. These Grand Bank groups were evident from 1984 through 1987 and in 1990. The central location of these groups, near the border of 3L with 3NO (see Fig. 1 for division locations), suggests either that these fish moved north from the southern half of the Grand Bank in 1984–1987 and 1990 or, perhaps more likely, that this group represents a substock of cod whose offshore and inshore movements are restricted to the Grand Bank (Templeman 1962; Lear 1984). These observations, coupled with the absence of high density aggregations south of Funk Island Bank in 1989, argue against the hypothesis that cod experienced southerly shifts in their distribution in 1984 and 1985 and in the years after 1988 as proposed by deYoung and Rose (1993) and Rose et al. (1994). Additional inconsistencies with the southern shift hypothesis are the observations that large groups of cod were located throughout the northernmost NAFO division (2J) in 1984, 1985, 1989, and 1991. If an unfavourable environment precipitated a southerly shift in cod distribution, one would have expected cod in the northernmost, and presumably least favourable, part of the range to have moved south. The spatial plots of high density aggregations indicate that this did not occur.

Temporal changes in the distribution of medium density tows appear to be less variable than those of high density tows (Fig. 8). The location of medium density aggregations on the Grand Bank provides additional support for the hypothesis that these fish are part of a substock whose movements are restricted to Grand Bank. There is evidence of medium density groups of cod on north–central Grand Bank every year from 1981 to 1992 (with the exception of 1991). There are few, if any, differences in the spatial distribution of medium density aggregations on north–central Grand Bank in the years 1983 through 1990.

Rather than reflecting dramatic temporal changes in location, the spatial distribution plots of high and medium density tows are indicative of stability rather than variability. Rose (1993) predicted that northern cod migration routes coincide with deep (greater than 300 m), warm water (2–3°C) trenches in the northeast Newfoundland Shelf. Depending on the sampling time in autumn, cod aggregations would be expected to be found either along or at the end of these migration routes. The three routes proposed by Rose (1993) would lead to cod aggregating on or near (*i*) Hamilton Bank, (*ii*) Funk Island and Belle Isle Banks, and (*iii*) at the terminus of the channel running east from the Bonavista Peninsula. The spatial plots of high, and to a lesser degree medium, density tows from 1981 to 1992 tend to support Rose's (1993) prediction. Cod were highly aggregated on or near Hamilton Bank every year from 1981 to 1989 and in 1991, on Belle Isle and Funk Island Banks every year except 1985 and 1992, and at the eastern terminus of the Bonavista channel in 1981, 1984–1986, 1988, and 1990–1992 (Fig. 7). To these three areas can be added a fourth, north–central Grand Bank, where cod were densely aggregated between 1984 and 1987 and in 1990.

The spatial distribution of medium density aggregations is

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Fig. 7. Locations of high biomass (>500 kg) research survey tows of northern cod between 1981 and 1992. Note that in 1991 and 1992 sampling effort in the south was almost three times that elsewhere in the survey area (see text for details). Depth contours are 200 m (broken line) and 400 m (solid line).

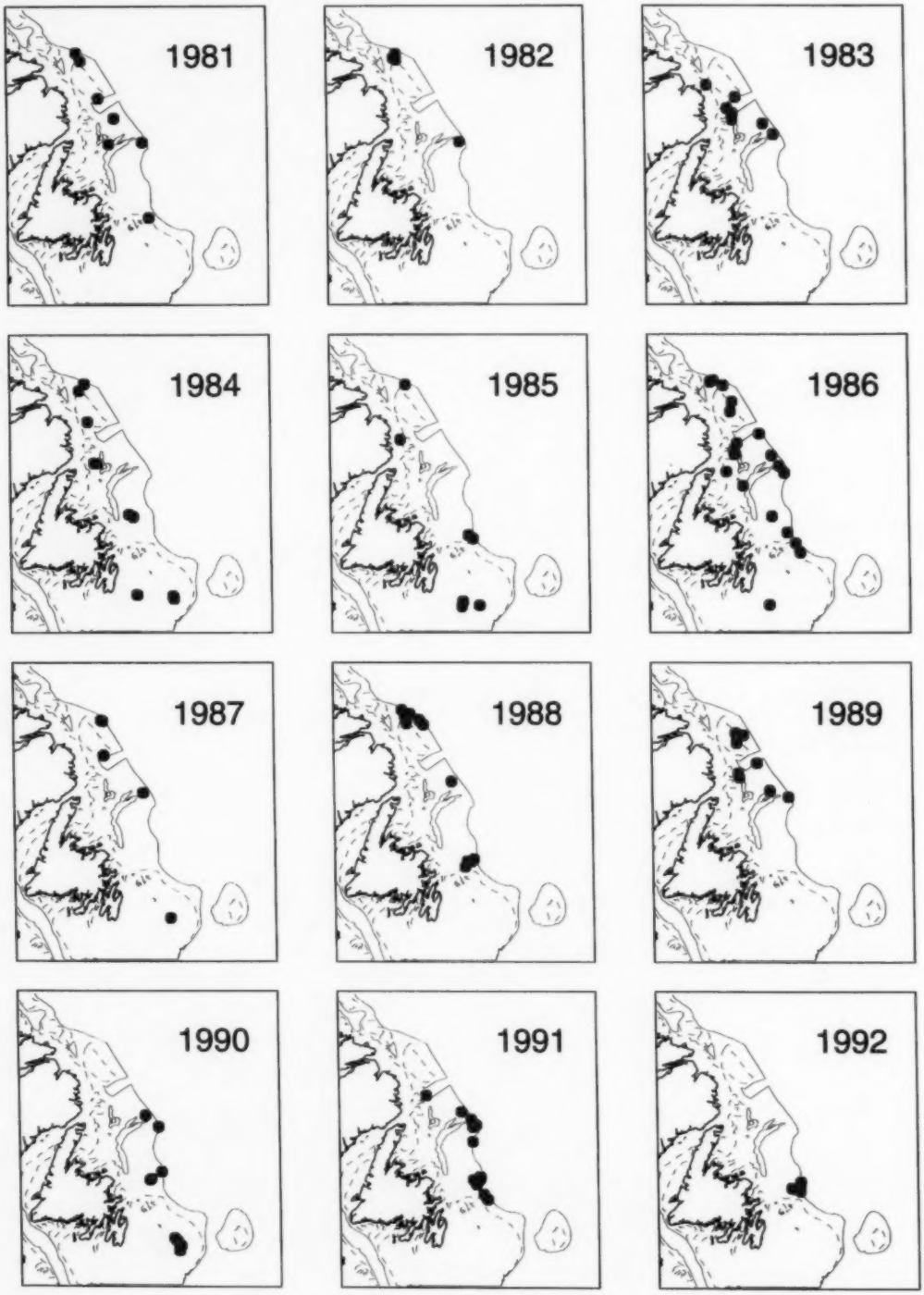

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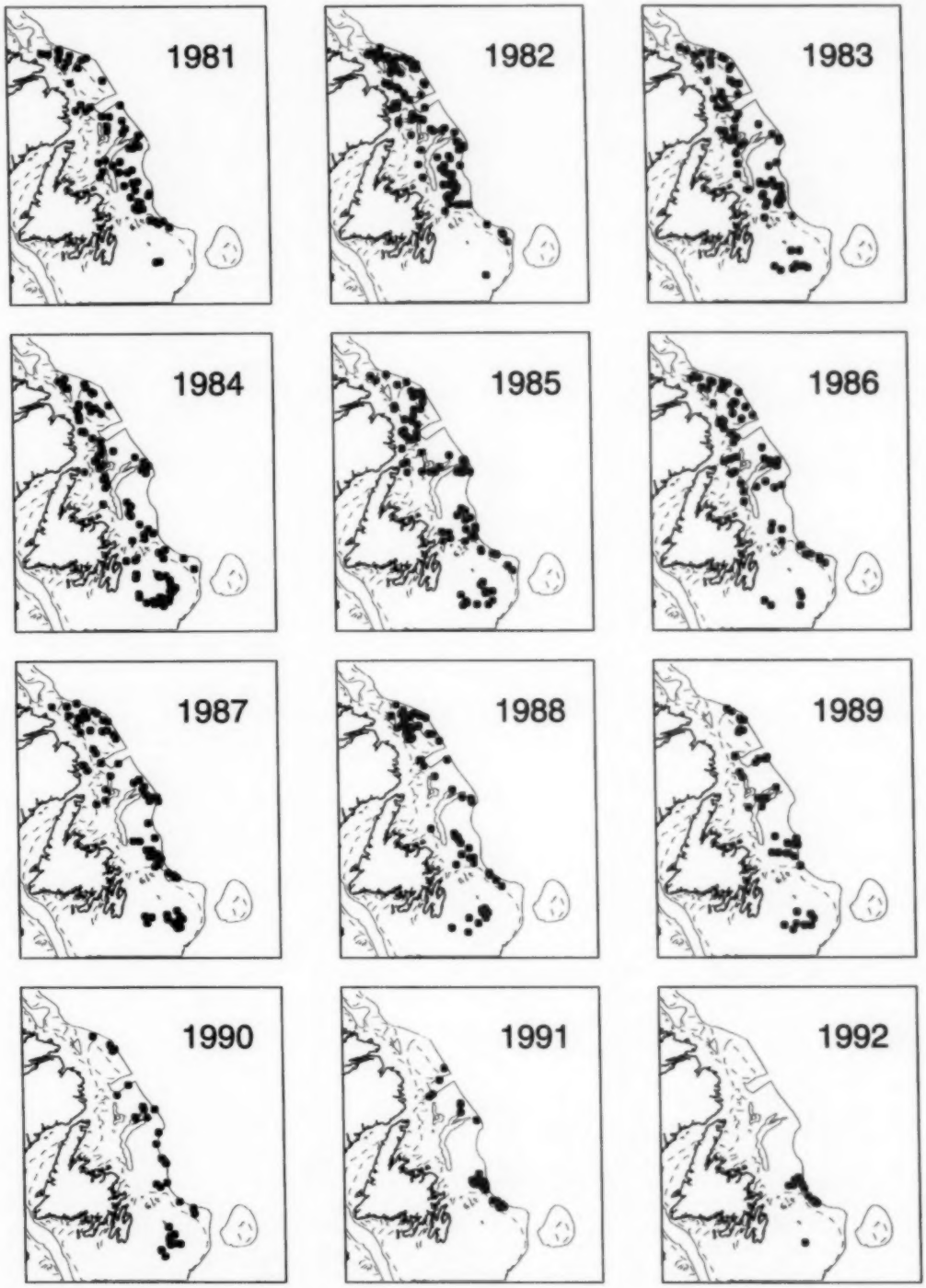
Fig. 8. Locations of medium biomass (100–500 kg) research survey tows of northern cod between 1981 and 1992. Note that in 1991 and 1992 sampling effort in the south was almost three times that elsewhere in the survey area (see text for details). Depth contours are 200 m (broken line) and 400 m (solid line).



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consistent with that of high density aggregations (Fig. 8). Medium density tows were sampled in each of the four offshore regions until 1991 when none were observed on north–central Grand Bank and in 1992 when the Bonavista and north–central Grand Bank concentrations were the only ones observed. Temporal changes in the distribution of medium density tows are not consistent with the hypothesis that cod shifted their distribution southward in 1989 and 1990. Rather, the spatial changes in the late 1980s and early 1990s are consistent with a gradual reduction in cod beginning in the north.

When evaluating the temporal trends in the location of high and medium density tows in Figs. 7–8, note that significant increases in survey sampling effort (number of tows per stratum) occurred in 3L in 1991 and 1992 relative to the remainder of the survey area. The increase was largely restricted to the eastern edge of the boundary between 3K and 3L, near the end of a channel that extends westward to the Bonavista Peninsula, and included strata 346, 366, 368, and 369 in 3L and 638 in 3K. These strata correspond roughly to the six southernmost high density tow locations in 1991 and all such locations in 1992 in Fig. 7. Compared with the average number of tows per stratum from 1981 to 1990, sampling effort in 1991 and 1992 for all but these five strata increased by an average 25%. For the five strata on the eastern edge of the 3K–3L boundary, the number of tows per stratum in 1991 and 1992 more than trebled. Thus, the relatively high number of medium and high density tows near the eastern edge of the 3K–3L boundary in 1991 and 1992, i.e., the southernmost high and medium density tows north of the Grand Banks in 1991 and 1992 (Figs. 7–8), reflects sampling effort rather than a southward movement of individuals. Given the increase in sampling effort that occurred in these areas relative to the remainder of the survey area, the number of tows in this area should be reduced by a factor of 3.17.

Confounding latitudinal changes in distribution with latitudinal changes in abundance

There is neither direct nor indirect evidence that cod shifted their distribution southward in 1989 or in 1990. (By a distributional shift, I am referring to spatial changes in abundance caused by active movement of fish.) The purported southward shift reported by previous authors appears, in part, to be an artifact of using changes in the arithmetic means biomass among NAFO divisions (2J, 3K, and 3L). Some of the reasons against the use of trends based on arithmetic means were discussed earlier. There are additional biases that need to be recognized. The main evidence for a southward change in distribution is the observation that the mean latitude of research survey biomass in 1989 and in later years is less than it was in previous years, with the exception of 1984 and 1985 (Rose et al. 1994). Uncritical use of mean latitudinal data overlooks the large influence that relatively small changes in density can have on such data. For example, as indicated by the spatial plots of high and medium density tows, cod are always located in abundance on north–central Grand Bank. There is no suggestion from Figs. 7 and 8 that cod in this region had moved from any other region. However, spurious latitudinal changes can be obtained in years in which these Grand Bank cod aggregated closer together or in years in which the research tows happened to sample the relatively few dense aggregations that exist there. The mean latitude of survey biomass in such years will appear to have declined despite the lack of latitudinal movement by cod.

The primary reason for not using changes in mean latitude of survey biomass or mean survey biomass per NAFO division as metrics of distributional change is that spatial changes in biomass can be confounded by numerical changes in abundance. It is just as valid, for example, to argue that declining mean latitude in biomass is indicative of a depletion of fish from high latitudes as it is indicative of a southward movement of fish. One means of accounting for changes in abundance when assessing possible changes in distribution is to examine spatio-temporal changes in the density composition of cod by NAFO division. The southward shift hypothesis proposes that cod shifted their distribution southward in 1989 and (or) 1990 (deYoung and Rose 1993; Rose et al. 1994). The conditions that prompt such a hypothesis are

$$n_{N(1989)} < n_{N(1988)} \text{ and } n_{S(1989)} > n_{S(1988)}$$

or

$$n_{N(1990)} < n_{N(1989)} \text{ and } n_{S(1990)} > n_{S(1989)}$$

where $n_{N(t)}$ and $n_{S(t)}$ represent abundance in the northern and southern of two adjacent NAFO divisions in year t. Bivariate plots of tows in adjacent divisions indicate that declines in the percentage of tows greater than 100 kg in 2J in 1989 and 1990 were accompanied by little change in the percentages in 3K in those years (Fig. 9A); this is also true of tows in 3K and 3L (Fig. 9B). For example, a southern shift of cod from 2J to 3K between 1989 and 1990 would have placed the 1990 datum in the upper left portion of Fig. 9A.

The lack of divisional shifts in the percentage of tows indicated in Fig. 9 argues against the southward shift hypothesis. The strong association between cod biomass in 2J and that in 3K suggests that a common factor influences fish abundance in these divisions (Fig. 9). The independence of cod biomass in 3K with that in 3L may indicate that the factors influencing abundance in these regions are dissimilar. This is consistent with the hypothesis, evident from tagging data (Templeman 1962; Lear 1984) and genetic analyses (Bentzen et al. 1997), that cod in 3L are of a different stock than those in 2J and 3K.

Latitudinal differences in growth rate and fishing mortality

Divisional comparisons of weight-at-age data for northern cod do not support the southward shift hypothesis. If cod had moved south in large numbers in 1989 or in 1990, such a massive distributional change should have been evident in the age-specific weights of cod sampled during the surveys. Cod in 2J have lower weights at age than do cod in 3K, which correspondingly have lower weights at age than cod in 3L (Templeman 1965). Combining age-specific weights for cod aged 6 through 9 years (the dominant ages in the surveys) from 1981 to 1988 (Bishop et al. 1994), the average size of cod in 2J is 83% that of cod in 3K, which are 88% that of cod in 3L (Table 1). If 2J cod had moved south into 3K, or 3K cod south into 3L, in either 1989 or 1990, this should have resulted in a reduction in the difference in age-specific weights among divisions (unless one assumes that only the fastest growing individuals moved south in each year). That is, the ratio of age-specific weights for cod in 2J to those for cod in 3K, and similarly for cod in 3K compared with cod in 3L, should have

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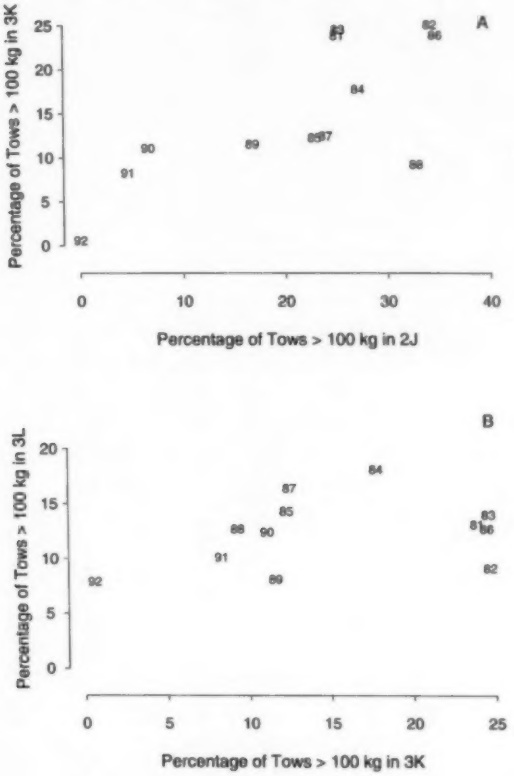
Fig. 9. Scatter plots of the percentage of research survey tows sampling more than 100 kg of northern cod from adjacent north–south NAFO divisions. Each datum is represented by the survey year. (A) Tows in 3K (south) versus tows in 2J (north). (B) Tows in 3L (south) versus tows in 3K (north).


A
Percentage of Tows > 100 kg in 3K
Percentage of Tows > 100 kg in 2J
B
Percentage of Tows > 100 kg in 3L
Percentage of Tows > 100 kg in 3K

approached unity in 1989 and 1990. Instead, the relative difference in age-specific weights for cod in 2J and 3K in 1989 and 1990 was unchanged from the 1981–1988 average whereas the ratio of age-specific weights in 3K to cod in 3L declined rather than increased (Table 1).

It has been argued that the declining abundance in the northern part of the northern cod's range cannot be attributed to fishing (deYoung and Rose 1993; Rose et al. 1994). This argument is based on the observation that fishing effort (number of trawling hours) is typically higher in 3L than it is in 2J and 3K. The assumption that number of trawling hours in a given NAFO division is directly proportional to the fishing mortality experienced by cod that are located in that division in late autumn is unwarranted because of cod movement among divisions and because of the exposure of cod to fishing mortality from gear other than otter trawls. For example, on the basis of tagging studies, it is clear that cod that reside in 2J during late autumn and early winter migrate across the northeast Newfoundland Shelf to inshore waters in 2J, 3K, and 3L (Lear 1984). Tagging studies also indicate that extremely high fishing mortality can be exerted by the inshore fishery (Myers et al. 1997).

Additional evidence that the southward decline in cod abundance represents depletion owing to fishing rather than a shift in abundance has been forthcoming from analyses of tagging experiments and from genetic analyses. Myers et al. (1995*a*) reported that fishing mortalities were higher in the north than they were in the south. This is consistent with the hypothesis that the decline of cod in 2J prior to their disappearance in 3K and 3L was due to fishing. Temporal changes in the proportion of tag recoveries from cod tagged in 3K that occurred on offshore banks south of 3K do not support the southward shift hypothesis. Of cod tagged in 3K between 1985 and 1989, a smaller percentage was recovered south of 3K (median 3.9%) compared with cod tagged in 3K between 1979 and 1984 (median 6.9%) (Myers et al. 1995*a*). Consistent with the hypothesis that northern cod have not significantly shifted their distribution since the late 1980s is the observation from microsatellite DNA analyses that the spatial distribution of cod genotypes in the 1960s, 1970s, 1980s, and 1990s has remained constant through time (R. Doyle, Department of Biology, Dalhousie University, Halifax, N.S., unpublished data).

Table 1. Average mass (kg) for cod aged 6–9 years sampled in the autumn research surveys conducted in NAFO divisions 2J, 3K, and 3L in selected years from 1981 to 1990.

NAFO division	1981–1988	1989	1990
2J	2.10	1.71	1.65
3K	2.52	2.12	1.98
3L	2.87	2.67	2.88
2J/3K	0.83	0.81	0.83
3K/3L	0.88	0.79	0.69

Nested aggregation model for the spatial decline of northern cod

The density composition data (Fig. 2) and the spatial distribution of medium and high density research survey tows (Figs. 7–8) suggest that the distribution of northern cod in late autumn and winter can be characterized by a small but temporally constant number of high density aggregations (Fig. 10; the time scale associated with this nested aggregation model and the percentage of space occupied by high, medium, and low densities of cod correspond with those of the density composition model given in Fig. 3). I have assumed here that aggregations can be modelled as circles, a shape consistent with field observations based on hydroacoustic data (Rose 1993). I have assumed further that high density aggregations are encircled by medium density aggregations. One alternative model is that high and medium density aggregations are spatially distinct entities. However, the general, albeit coarse, correspondence between the distribution of high and medium density tows (Figs. 7–8) suggests that the model of nested aggregations is not unreasonable. Implicit in the nested aggregation model is the assumption that there are fitness advantages to being members of large groups (e.g., protection from predators, increased probability of locating prey; Pitcher and Parrish 1993). All individuals may be prevented from being members of high density aggregations because of aggressive interactions (these can be very intense under certain conditions, e.g., spawning; Brawn 1961; J. Hutchings and T. Bishop, unpublished data). Alternatively, the proportion of the cod

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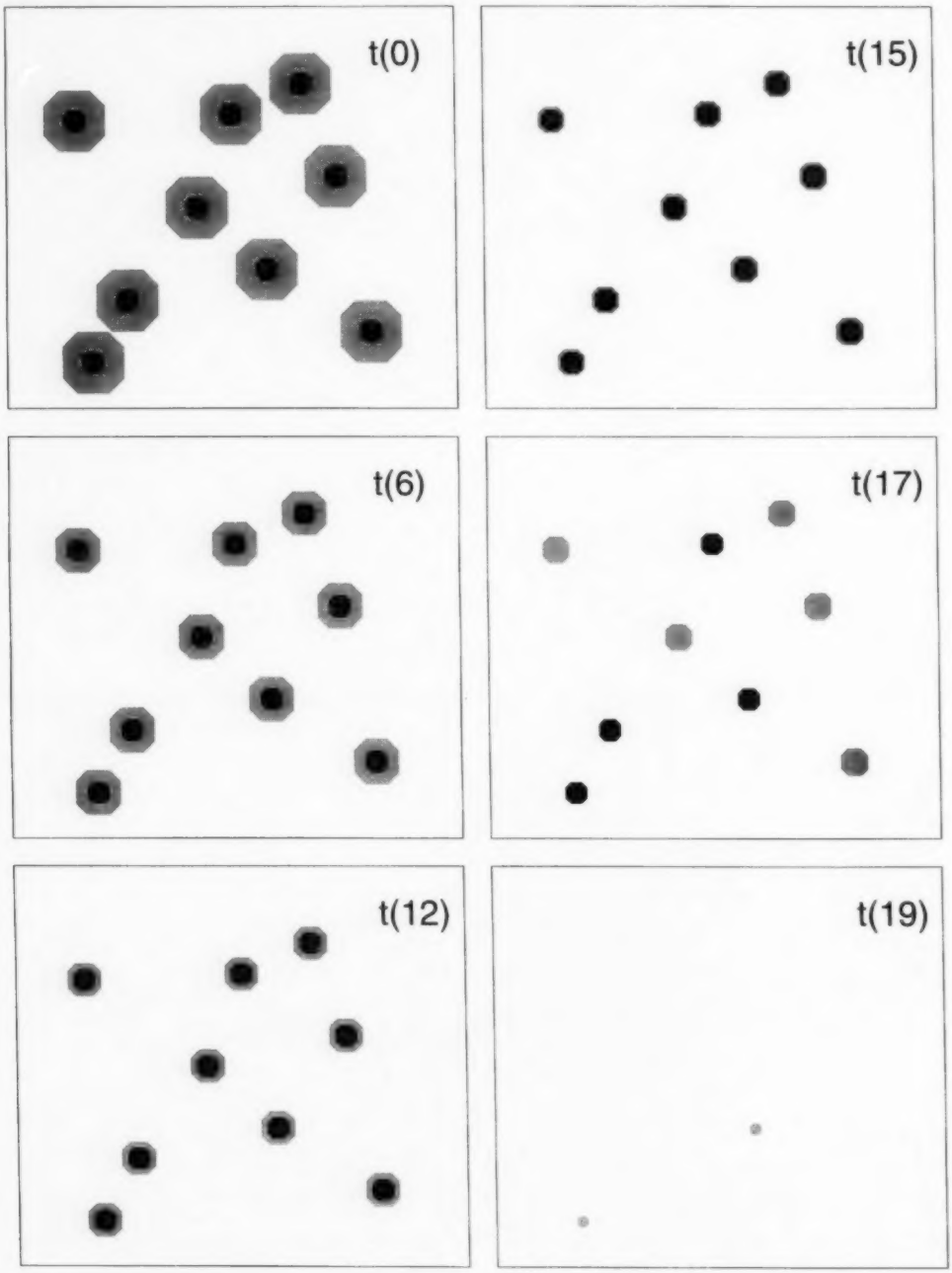
Fig. 10. Nested composition model describing changes in the density composition of the northern cod stock with declining stock biomass. High (>500 kg/tow), medium (100–500 kg/tow), and low (<100 kg/tow) densities of cod are distinguished by black, grey, and white areas, respectively. The percentage of area in each time square corresponding to each density category corresponds to those described in the density composition model (see Fig. 3).



stock occupying aggregations of different density may follow an ideal free distribution (Fretwell and Lucas 1970).

Fishing strategies exploit the spatial aggregations of cod by concentrating effort where catch rates are highest. Thus, mobile trawlers fishing for northern cod concentrate their effort during winter on the high density aggregations (Warner 1977;

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Hutchings and Myers 1994*a*; Kulka et al. 1995). Fishers using fixed gear such as gill nets and cod traps are presumably exploiting cod at medium and low densities after they have dispersed along the coast following their spring inshore migration.

Research survey data indicate that the percentage of high density tows remains constant over time. This suggests that despite being "fished down" by trawlers, high density aggregations may be able to maintain their numerical integrity by recruiting cod from medium density areas. Again this is predicated on the reasonable assumption that there are fitness advantages to being part of a high density aggregation and that this is always the preferred behavioural strategy of cod. Temporal patterns in trawler and fixed-gear catch rates can be explained by this model. As stock biomass declines, trawler catch rates will remain constant as long as biomass is sufficiently high to allow cod to form high density aggregations. In constrast, as trawlers fish down high density aggregations, there will be fewer fish migrating inshore and dispersing along the coast, resulting in a steady decline in fixed-gear catch rates (Fig. 5A; Hutchings and Myers 1994*a*). This would lead to a time lag of 1 year between the fishing down of the high density aggregations and recruitment from medium density aggregations. Thus, as cod abundance declines, the areal extent of high density groups remains unchanged, whereas that of the medium and low density areas would be expected to decline and increase, respectively. Somewhat similar observations have been made for southern Gulf of St. Lawrence cod for which the area containing 50% of the population remained constant despite declines in stock abundance (Swain and Sinclair 1994).

When cod abundance has declined to a level at which the recruitment of cod from medium density areas is unable to replace those fished from high density aggregations (this would correspond to time t^* in eqs. 1–3 at which the threshold percentage of medium density areas had been reached), the high density aggregations begin to disappear and catch rates in high density areas begin to decline. Prior to time t^*, as stock biomass declines, fixed-gear fisheries exploiting cod at medium and low densities would be predicted to experience declining catch rates while trawler catch rates remained high. Perhaps most importantly, after several years of declining fish abundance in medium density areas, the nested aggregation model predicts that the areal extent of cod will become limited to a few, highly dense aggregations. Again, this is consistent with empirical data. Between 1989 and 1991, 50–60% of the estimated survey biomass of northern cod was located in these few highly dense groups (i.e., >500 kg/tow) compared with an average 21% from 1981 to 1985, rendering the stock increasingly vulnerable to mobile fishing gear. At constant catches (the TAC for northern cod increased from 1981 to 1988 before declining slightly from 1989 to 1991), the density composition model predicted that after several years of increasing fishing mortality, F would increase rapidly. This temporal pattern in F has also been estimated by VPA (Bishop et al. 1994) and by tagging data (Myers et al. 1995*a*).

The spatial model of northern cod aggregations presented here (*i*) provides a framework for explaining changes in the density composition of cod in the 1980s and early 1990s, (*ii*) provides an explanation for the observed divergence in fixed-gear and mobile-gear catch rates that began in the mid 1980s (Hutchings and Myers 1994*a*), (*iii*) accounts for the curvilinear relationship between trawler catch rate and harvestable biomass of northern cod (Hutchings and Myers 1994*a*), and (*iv*) suggests that the behaviour of forming high density aggregations increases the vulnerability of northern cod to fishing gear as stock size declines, thus providing one reason why fishing mortality increased so rapidly in the late 1980s and early 1990s. The nested aggregation model represents one possible framework for the changes in stock density composition documented in the research survey data. It may be possible to use the survey data to estimate the probability with which alternative spatial models of cod aggregation are consistent with these data.

Review of alternative hypotheses for the collapse of northern cod

From the time that a moratorium was imposed on the commercial fishery for northern cod in July 1992, there has been considerable debate over the relative importance of environmental factors and fishing to the stock's collapse. Alternative hypotheses characterize the increase in mortality of cod throughout the 1980s and early 1990s as being largely a function of increasing natural mortality, increasing fishing mortality, or some combination thereof. Many of these hypotheses have been subjected to statistical analysis, others have not. These will be reviewed briefly to place the previously described density composition and nested aggregation models, and the insights they provide about the collapse of northern cod, in a broad context.

Decline of noncommercial species

The concomitant decline of some fish species not directly targeted by fishing has frequently been cited as evidence that ecosystem change contributed significantly to the stock's collapse (Atkinson 1993; Atkinson and Bennett 1994; Department of Fisheries and Oceans 1995; Fisheries Resource Conservation Council 1995). These declines have been described from trends in arithmetic mean biomass (Atkinson 1993; Gomes et al. 1995). As discussed earlier, there can be strong limitations to the extent to which trends based upon arithmetic means reflect actual changes in stock abundance. Some researchers examining changes in fish community structure on the northeast Newfoundland Shelf have identified bycatch fishing mortality as a dominant influence of abundance changes in noncommercial species on the northeast Newfoundland Shelf (Gomes et al. 1995; Haedrich 1995). Others disagree with this interpretation, particularly with regard to the decline of American plaice, *Hippoglossoides platessoides*, in division 2J. Brodie et al. (1995), for example, argue that fishing cannot account for the decline of 2J plaice because reported catches of plaice have been low and have never reached their TAC of 10 000 t in the 1980s. One implication of this conclusion is that changes in the environment caused the natural mortality of plaice to increase dramatically in the 1980s.

Data from research surveys and commercial catches suggest that the mortality of American plaice caught as bycatch in the northern cod fishery have been considerable. For example, research survey data collected throughout 2J from 1981 to 1992 indicate that, on average, 95.1% of all tows that caught cod also caught American plaice. This suggests that the habitats of cod and plaice overlap considerably and that the

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Table 2. Associations between the incidence of American plaice, *Hippoglossoides platessoides*, and Atlantic cod, *Gadus morhua*, in research survey tows in NAFO division 2J from 1981 to 1992.

Year	Tows of cod containing plaice (%)	Regression between plaice (y) and cod (x) biomass	p	Catches of 2J plaice (t)	
				Reported	Predicted
1981	100	$y = 0.055x - 1.10$	<0.001	96	1479
1982	92	$y = 0.076x + 0.63$	0.002	204	5115
1983	77	$y = 0.181x - 0.05$	<0.001	168	7500
1984	93	$y = 0.589x + 17.1$	<0.001	92	7076
1985	82	$y = -0.040x + 2.74$	0.70	34	0
1986	92	$y = 0.075x + 2.76$	0.003	100	901
1987	71	$y = 0.172x + 2.72$	0.44	239	7196
1988	83	$y = 0.025x + 0.35$	0.003	106	1037
1989	94	$y = 0.022x + 3.42$	0.20	3225	761
1990	83	$y = 0.070x + 1.04$	<0.001	991	1295
1991	100	$y = -0.011x + 5.15$	0.47	69	0
1992	90	$y = -0.533x + 2.22$	0.19	5	0

Note: Reported catches of plaice in 2J from Brodie et al. (1995). Predicted catches of plaice in 2J were calculated from the reported catches of 2J cod by trawlers (data in Bishop et al. 1994), using the regressions between plaice and cod for strata 222 through 233, inclusive, and stratum 236 (x and y are in kilograms).

probability of catching both species together in trawl gear is very high.

Annual regressions between plaice and cod biomass can be used to estimate the biomass of plaice caught as bycatch in the fishery for cod in 2J (the value for cod biomass being the reported catch of trawlers in 2J; Bishop et al. 1994). For this analysis, the tows on which the plaice–cod regressions were based were restricted to the area encompassed by the winter trawler fishery for northern cod (Wroblewski et al. 1995), i.e., strata 222 through 233, inclusive, and stratum 236 (Table 2). From 1981 to 1992, an annual average of 88.1% of all tows that caught cod in these strata also captured American plaice.

The predicted catches of American plaice in 2J exceed the reported catches every year between 1981 and 1990 except 1985 and 1989 (Table 2). Annually from 1981 to 1984, actual catches are predicted to have been more than one order of magnitude greater than reported catches; the discrepancies were lower between 1986 and 1990. The smaller differences between predicted and reported plaice catches in the late 1980s may be attributable to the increase in observer coverage of Canadian trawlers fishing for northern cod from 20% before 1987 to 100% thereafter (Wroblewski et al. 1995). Predicted plaice catches have been underestimated in this analysis because of discarding of northern cod and because of the omittance of other 2J groundfish fisheries. Elsewhere in the northwest Atlantic, high bycatches of American plaice in the cod fishery, a problem long recognized in the Gulf of St. Lawrence (e.g., Chouinard and Metuzals 1984), have prompted the industry to request that stricter regulatory measures be imposed to reduce this nontargeted fishing mortality (e.g., Atlantic Groundfish Advisory Committee 1992).

On the basis of the survey data, then, it is logical to assume that the catching of American plaice in commercial trawls fishing for cod would have been unavoidable and that the amount of plaice captured as bycatch would have increased with increasing catches of cod. The argument that fishing cannot explain the decline of 2J plaice because of low annual ratios of (reported catch)/(survey biomass), a proxy for exploitation rate (Brodie et al. 1995), needs to be re-evaluated in light of the differences in reported and actual plaice catches predicted from research survey data.

Poor recruitment to the fishery

Northern cod, and other northwest Atlantic cod stocks, were closed because of a scarcity of individuals of reproductive age, i.e., low spawner biomass. It has been proposed that a primary reason for this relative absence of spawners was an increase in the natural mortality of cod aged 1–3 years in the 1980s (deYoung and Rose 1993; Atkinson and Bennett 1994; Mann and Drinkwater 1994). This "poor recruitment" hypothesis has recently been examined for the six Canadian cod stocks for which fishing moratoria currently exist. Using research survey data, Myers et al. (1996*a*) tested the null hypothesis that recruitment of those year-classes that would have dominated the spawner biomass at the time of the moratoria (collapse recruitment) was not significantly different from the average annual recruitment preceding those year-classes (precollapse recruitment). This hypothesis could not be rejected for five of the six stocks examined. The only stock for which the precollapse recruitment exceeded the collapse recruitment was the southern Grand Bank stock (divisions 3N–3O), much of which exists outside Canada's 200-mile limit. Declining recruitment in southern Grand Bank cod in the late 1980s would appear to be the result of the fishing gear used by foreign trawlers that caught disproportionately high numbers of 2- and 3-year-old cod (Walsh et al. 1995). The conclusion that poor recruitment did not contribute to the collapse of cod has also been reached for the southern Gulf of St. Lawrence stock (Sinclair et al. 1995). Analyses that previously linked poor recruitment to environmental factors such as cold water temperature (deYoung and Rose 1993) and low salinity (Myers et al. 1993) have not been supported on re-examination of the data (Hutchings and Myers 1994*a*).

Increased predation by marine mammals

The influence of seal predation on the collapse of northwest Atlantic cod stocks is one area in which there is general agreement. Harp seals, *Phoca groenlandica*, which eat primarily 1- and 2-year-old Atlantic cod (Stenson et al. 1995), increased in

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abundance throughout the northwest Atlantic in the 1980s (Shelton et al. 1995). Increased predation of cod by seals should have resulted in reduced numbers of 3 year olds, i.e., recruits to the fishery in the 1980s. However, the lack of significant changes in mean recruitment between precollapse and collapse periods (see above) suggests that seals did not have a significant effect on the collapse of east coast cod stocks (Myers et al. 1996*a*). The hypothesis that seal predation was a major factor contributing to the decline of Atlantic cod has also been rejected for southern Gulf of St. Lawrence cod (divisions 4T–4Vn; Sinclair et al. 1995) and eastern Scotian Shelf cod (divisions 4Vs–4W; Mohn and Bowen 1994).

It is not clear whether predation of 1- and 2-year-old cod by seals is retarding the rate of recovery of the collapsed stocks. The only independent source of abundance data on cod aged 1 and 2 years, the research survey data, provides no evidence that mortality at these ages has changed significantly since the early 1980s (A. Sinclair, R. Myers, and J. Hutchings, unpublished data). Using a deterministic, age-structured population dynamics model, Mohn and Bowen (1994) estimated that the mortality of juvenile cod on the eastern Scotian Shelf caused by predation by grey seals, *Halichoerus grypus*, may have increased fourfold between the early 1980s and the early 1990s. The statistical power of both analyses is relatively low. Because of the paucity of abundance and mortality data on juvenile cod, there are no empirical data to support the hypothesis that the influence of seal predation on the recovery of northern cod is disproportionately greater than the influence on recruitment of other predators of juvenile cod, historically low levels of cod spawner biomass, competitors of cod for food and space (e.g., possibly Arctic cod, *Boreogadus saida*), and natural environmental variability in recruitment.

Reported and unreported catches of young cod

Recent work suggests that increased discarding of prereproductive individuals contributed to the rapidity with which the northwest Atlantic cod stocks collapsed. Myers et al. (1996*a*) noted that VPA estimates of recruitment declined with time at a significantly greater rate than did recruitment estimated from research surveys. Given that VPA abundance data are based on reported catches, Myers et al. (1996*a*) hypothesized that the negatively biased VPA estimates of recruitment could be explained by temporally increasing levels of discarding and catch misreporting of pre-reproductive cod. Their hypothesis was supported by the observation that the rate of increase in mortality of 3-year-old cod, as estimated from research surveys, with VPA-based fishing mortality of cod fully recruited to the fishery was significantly greater than the rate of increase in 3-year-old cod mortality estimated by VPA. The hypothesis is based on the premise that as stock abundance declines, it becomes increasingly difficult to catch large fish. The increased effort required to maintain catch levels would result in increased catches of undersized fish, which cannot be sold, that are then discarded and then excluded from the catch data upon which VPA data are based, leading to negatively biased estimates of past abundance. A limited number of interviews with trawlermen and fishery observers (Eco-Research Programme, Memorial University of Newfoundland, unpublished data) indicate that discard estimates for trawlers in the northern cod, purportedly less than 2% by weight in the late 1980s (Kulka 1989), have been seriously underestimated. Reports from Department of Fisheries and Oceans port technicians provide additional evidence that official trawler discard data for northern cod are negatively biased. One such report, dated 24 December 1990, noted that trawlers fishing northern cod in 2J were "having to catch 500K [thousand], discarding 300K undersize, to get 200K legal size 2J cod" (Angel et al. 1994, p. 102). Widespread catch misreporting and discarding have been reported for the trawler fisheries for the northern (Palmer and Sinclair 1996) and southern Gulf of St. Lawrence cod (Sinclair et al. 1995) and the eastern Scotian Shelf (Angel et al. 1994) cod fisheries.

The inshore trap fishery was another source of high fishing mortality on pre-reproductive cod. Trap selectivity studies indicate that the percentage of trap catches (by number) that comprised undersized cod (cod less than 41 cm, approximately 3–4 years old, are generally not marketable in Newfoundland) can range from as little as 25% (e.g., Cape Broyle in 1990) to as much as 87% (e.g., Torbay in 1991) (Brothers and Hollett 1991; Brothers and Peddle 1991). Estimates of discarding in the trap fishery have been obtained from interviews with 45 fishermen south of St. John's (Cape Broyle) west to Fogo, Notre Dame Bay (J.A. Hutchings and M. Ferguson, unpublished data). All fishermen interviewed indicated that they discarded undersized cod from their traps. By weight, the average estimated discarding rate per trap haul averaged 17% and ranged from 10 to 50%. Comparing discarding rates between the early and late 1980s, inshore fishermen in some areas indicated that discarding may have more than doubled during this period because of the decline in abundance of larger, older cod.

The decline in availability of larger cod in the late 1980s and early 1990s is evident from the reported catch statistics for the northern cod fishery in 1990 and 1991 (Bishop et al. 1993). Until 1990, the decline of northern cod was partially masked in the commercial catch statistics by the extremely strong 1981 year-class (this cohort produced the highest absolute recruitment since 1968). This is evident from the observation that the catches produced by this year-class in the late 1980s were the highest since the early and mid-1970s. For example, the numerical catch of 5-year-old cod in 1986 was the highest since 1973, the 1987 and 1988 catches of 6 and 7 year olds were the highest since 1974, and the 1989 catch of 8 year olds was the highest since 1975. By contrast, the 1990 and 1991 commercial landings were dominated by cod aged 3–5 years. The 1986 year-class produced the largest catches of 4 and 5 year olds since 1977 and 1973, respectively; in 1991, the 1987 year-class contributed the highest catches of 3 year olds since 1976. One direct consequence of this overexploitation of 3, 4, and 5 year olds in 1990, 1991, and 1992 is that northern cod spawner biomass has remained at historically low levels into the mid-1990s despite the fishing moratorium.

Environmental–ecosystem changes and the role of fishing in the collapse of northern cod

Hypotheses linking the collapse of northern cod to environmental or ecosystem change bear the implicit assumption that environmental conditions in the late 1980s and early 1990s were temporally anomalous, given that collapses of the magnitude documented in 1992 have never been previously recorded. A comparison of various environmental indices on a decadal and on a century time scale indicate that the environ-

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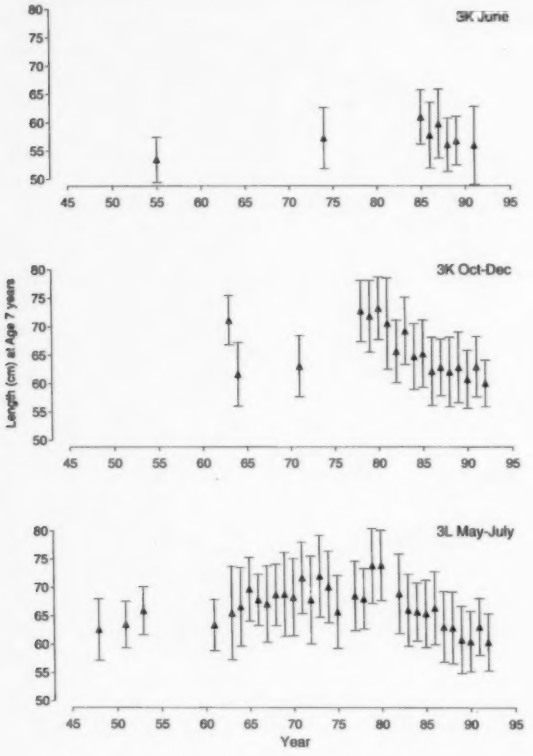
Fig. 11. Long-term temporal changes in mean length-at-age for northern cod aged 7 years. Data are for research cruises conducted in June in 3K (1955 to 1991), between October and December in 3K (1963 to 1992), and from May through July in 3L (1948 to 1992). Error bars represent one standard deviation.



mental conditions experienced by northern cod since the late 1980s have clearly been experienced by the stock in the past. For example, water temperatures recorded on the northeast Newfoundland Shelf were not temporally anomalous in the early 1990s. The same is true of water temperatures in the Gulf of St. Lawrence (Sinclair et al. 1995), Cabot Strait, and the Scotian Shelf (Drinkwater et al. 1995). Although low, the depth-averaged water temperatures recorded at a hydrographic station east of St. John's (station 27) in 1991 did not differ statistically from those in 1972–1974 and 1984–1985; bottom and near-bottom temperatures at this station were, in fact, lower in these two previous time periods (Hutchings and Myers 1994*a*). In addition, the volume of water less than 0°C (the cold intermediate layer or CIL) in 1990 and 1991 off southern Labrador was actually lower than it was in 1972 and in 1985; the 1990–1991 CIL volume further south off Cape Bonavista was also less than the CIL volume in 1985 and comparable with that recorded in this area in 1972 (Colbourne 1995).

Data available over a considerably longer time scale (e.g., water temperatures on Grand Bank since 1910, ice clearance data from Labrador since 1800, air temperature data from St. John's since 1874, the latter two of which are significantly associated with water temperature at station 27) indicate that northern cod catches of the size that were not sustainable in the 1980s were sustainable in the 19th and early 20th centuries in an environment that was, on average, considerably colder (Hutchings and Myers 1994*a*). In addition to its rejection on statistical grounds (Hutchings and Myers 1994*a*), the hypothesis that cold water temperatures cause poor recruitment does not bear up well under the observation that recruitment of St. Pierre Bank cod (division 3Ps) in the 1980s was not affected by a massive 2°C decline in water temperatures, from an average of 1 to –1°C, in 1985 (January through June average temperature at 50 m; Hutchings and Myers 1994*b*); there is no evidence of a similar decline in temperature in 2J3KL (Hutchings and Myers 1994*a*; Colbourne 1995). The description of oceanographic conditions in the early 1990s as temporally anomalous is unjustified on the basis of time series of several water temperature data.

There is, to date, no direct evidence linking the decline of northern cod to changes in the environment or ecosystem. Any effect of temperature or salinity on recruitment can be rejected on statistical grounds (Hutchings and Myers 1994*a*). The null hypothesis that cod did not shift their distribution southward in 1989 or 1990 (one purported response to environmental–ecosystem change; deYoung and Rose 1993; Rose et al. 1994) cannot be rejected by empirical data. As discussed earlier, neither the research survey data, age-specific growth data, tagging experiments, or spatial-temporal analyses of DNA indicate that cod shifted their distribution southward in the late 1980s or early 1990s. Lilly (1995) reported that there is no evidence that average stomach fullness of cod declined before or in parallel with the decline of northern cod. This argues against the hypothesis that a severe decline in prey abundance contributed to the cod collapse (Atkinson and Bennett 1994).

The argument that the collapse of northern cod can be attributed to an interaction between overfishing and a deteriorating environment (reflected by declining age-specific body sizes) has suffered from Pauly's (1995) "shifting baseline syndrome of fisheries." Time series of weights at age used in such analyses almost always begin in the late 1970s or early 1980s (e.g., Bishop et al. 1994; Taggart et al. 1994); however, if the time series is extended to include age-specific body sizes over all years for which such data are available, it is apparent that it is the large age-specific body sizes of the late 1970s and early 1980s that are anomalous (Fig. 11). (The similarity in temporal trends within 3K and between 3K and 3L suggests that these data are not unduly biased by small-scale spatial differences in sampling.) The decline in weight at age observed through the 1980s may be attributable to the increased rate of harvesting of the fastest growers within each cohort. Consistent with this hypothesis are the observations that the high age-specific body sizes measured in the late 1970s and early 1980s coincided with the time period during which cod experienced the lowest offshore fishing mortality since the early 1960s (Bishop et al. 1994; Myers et al. 1995*a*). In addition, the physical oceanographic environment of the late 1970s and early 1980s (e.g., water temperature; Hutchings and Myers 1994*a*) was not notably favourable for growth. The inability to detect an environmental influence on cod mortality does not mean, of course, that the environment has no effect on cod production. It would seem reasonable, however, on the basis of available data for northern cod and its environment, to conclude that such an

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effect has been small relative to the influence of fishing mortality.

It has been suggested that the collapse of northern cod occurred suddenly over a single year, the spring of 1991 being the favoured time (Lear and Parsons 1993; Atkinson and Bennett 1994). As discussed earlier, there is good reason to believe that this perception can be partly attributed to the use of trends in arithmetic mean biomass as a primary indicator of stock abundance. Other metrics, such as the geometric mean biomass, the rank-based biomass trend, and the spatial distribution of cod biomass through 2J3KL (Figs. 6–8), indicate that the decline of northern cod was gradual rather than abrupt. The decline in the percentage composition of medium density research survey tows also clearly indicates a decline in cod abundance throughout the 1980s (Fig. 2). The decline in fixed-gear catch rates, evident since the mid–1980s, suggests that the stock had been decreasing since at least 1985 (Hutchings and Myers 1994*a*). The dramatic spatial shift in the gill-net fishery in the mid–1980s from inshore to offshore waters in response to declining catch rates is further evidence of a decline prior to the 1990s (Hutchings and Myers 1994*a*, 1995).

There is considerable evidence, then, to support the hypothesis that overfishing was the primary, if not sole, cause of the collapse of the northern cod. The northern cod decline occurred gradually throughout the mid-1980s (Hutchings and Myers 1994*a*; present paper) at the same time that offshore (Hutchings and Myers 1994*a*) and inshore (Neis et al., see footnote 2) fishing effort was increasing (a description of temporal changes in gear technology in the northern cod fishery is provided by Hutchings and Myers 1995). Northern cod were not sustainable at the age-specific rates of survival and fecundity experienced between 1985 and 1992 (Hutchings and Myers 1994*a*). Statistical analyses of catch-at-age data for northern cod provide no evidence of an increase in natural mortality of northern cod in 1991 (Myers and Cadigan 1995*a*, 1995*b*). One of the strongest sources of evidence that fishing mortality was excessive are the estimates of fishing mortality derived from tagging studies, which indicate that F on northern cod had exceeded 1.0 by the late 1980s (Myers et al. 1995*a*; 1997). The primary role of overfishing has also been accepted as an explanation for the collapse of southern Gulf of St. Lawrence cod for which trends in environmental factors and seal abundance were inconsistent with trends in cod mortality (Sinclair et al. 1995). The absence of depensation in stock-recruitment data from 125 commercially fished stocks is generally consistent with the hypothesis that stock collapses worldwide can be attributed primarily to fishing, rather than nonfishing, sources of mortality (Myers et al. 1995*b*).

Conclusions

The observation that over 90% of the world's fish stocks have been overexploited (Alverson et al. 1994) suggests either that the effects of fishing on exploited populations are constantly and consistently underestimated (i.e., poorly understood) or that their effects are well known but management agencies lack sufficient authority to control harvesting levels. In the case of Canadian cod stocks, the former condition would appear to hold true.

The inability to control fishing mortality that led to the collapse of Canada's groundfish stocks underscores a general lack of knowledge of the effects of different gear on the fishing mortality of targeted and nontargeted fish species. There is a need for a research initiative the objectives of which would be to quantify the size and age selectivities of all species caught by (*i*) gear type (e.g., otter trawl, gill net, trap, hook and line); (*ii*) gear characteristics (e.g., time period of gear usage, mesh size, trap and trawl design, type of hook and bait); (*iii*) time of day; (*iv*) time of year; and (*v*) geographical characteristics of the fishing grounds (e.g., proximity to land (inshore vs. offshore), bottom substrate (sand, boulder), water depth).

These data would allow one to assess a priori the risk of fishing mortality for nontargeted species by factors such as gear and region. For example, total allowable catches for species *a*, based on factors such as gear, vessel size, and season, could include bycatch probability estimates for species *b, c, d,* etc. From a conservation perspective, inclusion of such bycatch estimates in the quota would reduce the threat imposed on fish stocks by practises such as misreporting and dumping of catches. Comprehensive knowledge of the age- and size-specific vulnerabilities of targeted and nontargeted species to various gears (by mesh size, by season, by bottom substrate) would enable managers to select gear types, fishing seasons, and fishing areas in such a way as to minimize the capture of immature and (or) undersized, unmarketable individuals.

Failure to recognize the decline in northern cod biomass from the mid–1980s can be partly attributed to the use of catch rates by commercial trawlers as a metric of stock abundance (Baird et al. 1991). Although the research survey biomass index suggested that the stock had changed little in size between the early and late 1980s, the density composition data provided by the research surveys indicate that the survey biomass index was positively biased. I suggested earlier that this could be attributed to the use of annual arithmetic means to interpret temporal trends in biomass or to an overrepresentation of high density tows attributable to survey design bias. To increase the probability of detecting true changes in stock biomass, the use of alternative abundance estimators from the research survey data (e.g., geometric mean, rank-based means, median) should be encouraged.

It is also evident, for the northern cod fishery, that declining abundance was reflected by decreasing fixed-gear catch rates (Hutchings and Myers 1994*a*). These data clearly have the potential to provide an abundance metric for northern cod. The use of fixed-gear catch rates would also involve considerably closer interactions between fishers and the Department of Fisheries and Oceans than have existed in the past. One consequence of such stronger communications would be recognition of the scientific value of fishers' local knowledge (Neis 1992; Neis et al., see footnote 2). For example, local knowledge of the dates when fish are caught in fixed-gear locations can provide data on seasonal and directional movements of cod (and other fishes) in inshore waters, information for which there presently are no biological data. With respect to stock identification, fishers can provide information on temporal and spatial isolation of cod populations through identification of overwintering grounds (capture of cod through ice), spawning areas (presence of females and males in ripe and running condition), juvenile habitat (bycatch of young cod in caplin traps), morphological characteristics, and seasonal migrations. Thus, in addition to serving as partners in management strategies, inshore fishers can provide a wealth of information upon

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which hypotheses concerning the biology of cod can be formulated and tested and upon which juvenile and spawning areas (possible candidates for future protection) can be identified.

Seasonal or permanent closure of marine habitat (marine protected areas or MPAs; Shackell and Willison 1995) may increase the likelihood that commercially exploited fish populations can be maintained at sustainable levels. Seasonal fishery closures, particularly during periods of high catch rates, can act as a conservation measure by reducing overall fishing mortality. This was the basis for implementing seasonal spawning area closures for haddock, *Melanogrammus aeglefinus*, on Browns and Georges Banks southwest of Nova Scotia (Halliday 1987). Hutchings (1995) explored the usefulness of seasonal fishing closures on the northern cod fishery. He suggested that an offshore fishing ban on northern cod from January through May, the months coinciding with the highest catch rates associated with the fishing of spawning and prespawning aggregations, would conform to historical fishing patterns in this fishery and would re-establish the temporal and spatial refuges experienced by this stock prior to the 1950s.

Seasonal MPAs may have considerable merit when assessed against the effectiveness of other forms of regulatory control such as bycatch limitations and catch quotas. Enforcement of areal closures would be more cost effective and certainly easier to enforce than quota restrictions, compliance being determined by an absence of vessels fishing for cod. Although it has not been demonstrated that the prohibition of trawling of spawning northern cod is of intrinsic biological benefit, such a benefit cannot be justifiably discounted. If trawling disrupts the complex behavioural dominance hierarchies evident during spawning (Brawn 1961; J.A. Hutchings and T. Bishop, unpublished data), such fishing activity may reduce the number of matings per individual and deleteriously influence recruitment. It may be noteworthy, for example, that the exceptionally strong year-classes of northern cod between 1978 and 1981, the highest recruitment since the late 1960s (Bishop et al. 1994), coincided with the lowest level of trawling effort observed since the late 1950s (Hutchings and Myers 1994*a*, 1995). Despite its clear management implications, research on the effects of trawling on cod reproductive behaviour and fertilization success has yet to be undertaken.

The changes in density composition of northern cod documented here may provide useful and unambiguous criteria for re-opening the fishery. These data suggest that the re-opening of the fishery should not be considered until the percentage of high density autumn research survey tows (i.e., tows catching more than 500 kg of cod) reaches 2% of the total (Fig. 2). Once this criterion has been attained, the percentage of medium density tows (tows catching between 100 and 500 kg) should be allowed to increase to at least 20%. This density composition of northern cod would correspond roughly to the size of the stock in the early 1980s (Fig. 2) and should safely permit an annual harvest of northern cod of 100 000 to 150 000 t. One implicit assumption in using this criterion is that the density-dependent changes in the distribution of cod during stock recovery will mirror those observed during the stock decline. Density criteria could then be used in conjunction with a criterion stipulating that the fishery remain closed until the age-frequency distribution of cod sampled in the research surveys matched those observed during a period of comparatively high cod abundance.

There may be considerable merit in examining research survey data sets from other Atlantic cod stocks to model density-dependent changes in spatial structure (e.g., Myers and Stokes 1989; Swain and Wade 1993; Swain and Sinclair 1994), to assess the extent to which cod spatial structure can influence vulnerability to overexploitation, and to determine whether the density composition data for other stocks suggest criteria for re-opening these fisheries similar to those proposed for the northern cod fishery.

When will northern cod recover? This depends on one's definition of what constitutes a recovery. Population biologists would argue that a recovery would constitute a return of northern cod to its historical level of abundance. On the basis of the earliest available data from the early 1960s, such a recovery can be said to have occurred when spawner biomass exceeds 1.5 million t (it was probably less than 50 000 t in 1994) and recruitment exceeds 1 billion 3-year-old cod (Hutchings and Myers 1994*a*). However, the time scale for such a recovery unquestionably numbers in the decades and socioeconomic and political pressures (notwithstanding potentially important biological changes in the relative abundance of other species) will not permit such a period of time to elapse before the fishery is re-opened.

In the interim, it is critical that every effort be made to answer the dominant question that emerges from the review presented herein and on which future fisheries research, and the prevention of future fishery collapses, arguably hinges: What are the effects of fishing on the behaviour, life history, and population biology of exploited fishes?

Acknowledgements

The research was supported by a Natural Sciences and Engineering Research Council of Canada research grant and by the Northern Cod Science Programme. The northern cod survey data used in the present paper were obtained by Department of Fisheries and Oceans (DFO) staff in the Groundfish Division, Newfoundland Region, St. John's, Newfoundland. Funding for the fisher interviews referred to herein was provided by a Social Sciences and Humanities Research Council of Canada strategic grant to Larry Felt (Memorial University of Newfoundland, MUN), by a Tri-Council Eco-Research Project grant to MUN, and by DFO. The author thanks Doug Swain, Noel Cadigan, and Ram Myers for discussion and is grateful to Noel Cadigan, Gordon Mertz, Ram Myers, Tony Pitcher, Doug Swain, and Carl Walters for comments and criticism on an earlier version of the manuscript.

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Analysis of spatial structure in fish distribution using commercial catch and effort data from the New Zealand hoki fishery

Marianne Vignaux

Abstract: A linear model was fitted to commercial catch per unit effort data in the spawning hoki trawl fishery off the west coast of the South Island of New Zealand. Correlations in the residuals from this model extend out to 11 nautical miles and persist for periods of up to 14 days, suggesting that there is patchiness in the fish distribution on this spatial and temporal scale. Simulation studies show that this method can detect patchiness in the density distribution even when the trawl shots are comparable in length with the size of the patches, and that the extent of the correlations correctly estimates the size of the patches.

Résumé : Un modèle linéaire a été adapté aux données de prises commerciales par unité d'effort liées à la pêche au chalut de hokis reproducteurs sur la côte ouest de l'île sud, Nouvelle-Zélande. Les corrélations dans les résidus de ce modèle s'observent sur une distance de 11 milles marins et durant une période pouvant atteindre 14 jours, ce qui porte à croire que la distribution des poissons sur cette échelle spatio-temporelle est irrégulière. Des études de simulation révèlent que cette méthode permet de détecter l'irrégularité de la distribution de la densité même lorsque la longueur des traits de chalut correspond à celle des taches et que l'étendue des corrélations permet d'estimer correctement la taille des taches. [Traduit par la Rédaction]

Introduction

Standardized catch per unit effort (CPUE) analyses (Gavaris 1980) are commonly used in assessing fish stocks. They are usually used to obtain an index of relative abundance of a stock, although it is widely appreciated that there are many reasons why CPUE need not change linearly with abundance (Cooke 1985), and indeed when stock size has been measured independently, CPUE has usually turned out not to be an index of abundance, and in some cases to be dangerously misleading (Peterman and Steer 1981; Shardlow 1993; Butterworth 1980).

One of the factors identified by Cooke (1985) affecting the relationship between CPUE and abundance was the spatial distribution of the population. Similarly, Hilborn and Walters (1987) pointed out that the distribution of the fish and hence the distribution of fishing effort are essential, but mostly undetermined, ingredients in understanding the relationship between catch rate and abundance.

Several papers have described the spatial structure of fish populations in terms of the correlation structure (often expressed in geostatistical notation as a semivariogram) of data from trawl or acoustics surveys. Sullivan (1991) found that there was no significant correlation in acoustics measurements of the density of Alaskan walleye pollock at separations of more than about half a degree (about 30 nautical miles). Simard et al. (1992) found that in trawl survey data of Gulf of St. Lawrence shrimp, there was spatial autocorrelation suggesting mesoscale patches of about 30 km diameter (about 16 nautical miles). The sampling grid was too coarse to confirm this, however. Polacheck and Volstad (1993) studied haddock in Georges Bank and Gulf of Maine trawl surveys and found that the scale parameter in the exponentially declining function of correlation was about 9.53, so that correlation persisted out to about 9 nautical miles. Petitgas (1993) studied an acoustic survey of spring-spawning herring off the Norwegian coast and found that in all directions the extent of correlation was about 7 nautical miles.

CPUE data have the advantage over research trawl or acoustics surveys of having a much larger and richer data set, with many vessels providing data for a much longer period. However, because the data come from many different vessels with different catching abilities fishing under a range of different conditions, nearby tows are unlikely to be comparable. Therefore, in this work, a linear model was fitted to the CPUE data, to remove as much as possible of the variability owing to vessel differences and seasonal or time of day effects.

In principle, spatial or temporal correlations in the residuals from such a model could indicate spatial and temporal structure in the distribution of fish. As far as the author knows, such correlations have not previously been reported in data from commercial CPUE.

Background to the fishery

Hoki (*Macruronus novaezelandiae*) are widely distributed in New Zealand waters. The largest stock, the western stock, is thought to have feeding grounds in the sub-Antarctic region south of New Zealand (Fig. 1) and to migrate to the west coast of the South Island to spawn in July and August each year (Sullivan et al. 1995).

There is an intense trawl fishery on the spawning aggrega-

Received March 29, 1995. Accepted November 20, 1995.
J12844

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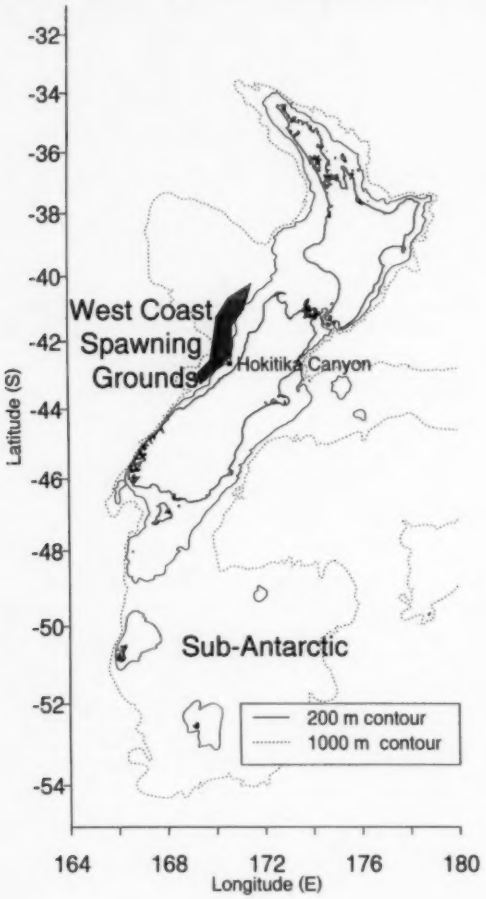
Fig. 1. Map showing the feeding grounds (sub-Antarctic) and the spawning grounds (west coast spawning grounds) of the western stock of New Zealand hoki (*Macruronus novaezelandiae*).



tions, consisting mainly of large foreign vessels chartered by New Zealand companies. In 1993 there were 61 vessels in the fishery, in an area about 130 nautical miles long by 30 nautical miles wide. The vessels ranged from 25 to 105 m overall length, and came from a number of countries, including Japan, former Soviet countries, Korea, and Norway.

Vessels over 43 m length are prohibited from fishing within 25 nautical miles of the coast. This line truncates the head of the Hokitika canyon, at the southern end of the spawning area. Most of the fleet are therefore prevented from fishing at the head of this canyon, which can be where some of the most dense aggregations are to be found.

Mid-water or bottom trawl nets are used in depths of 300–700 m. All vessels fishing in New Zealand waters are required to report both their catch and effort on forms submitted to the Ministry of Agriculture and Fisheries. Most of the larger vessels fill in detailed forms known as Trawl Catch Effort and Processing Returns in which details about each tow are recorded.

The western hoki stock is assessed using three indices: a time series of trawl surveys on the feeding grounds in the sub-Antarctic in November–December, a time series of acoustic surveys in the spawning fishery (July–August), and a time series of standardized CPUE indices from the commercial fishery (July–August). The CPUE indices are standardized using linear models. These three indices are used in a stock reduction model (Sullivan et al. 1995) to assess the current biomass and the virgin biomass before fishing. The biomass in 1995 of the western stock was estimated in 1994 to be 1.54 million tonnes, with a virgin biomass of 1.69 million tonnes and a long term yield of about 185 000 $t \cdot yr^{-1}$. In 1994 there was an annual total allowable commercial catch (TACC) on hoki in the entire exclusive economic zone (EEZ) of 202 000 t, allocated by individual transferrable quota (ITQ).

Linear modelling of the catch and effort data

Because catch and effort data come from many different vessels fishing under different conditions, nearby tows cannot be compared directly. Therefore, a linear model was fitted to the CPUE data, attempting to remove as much as possible of the variability owing to vessel differences and seasonal or time of day effects. The residuals from this linear model are the data that are analysed for spatial and temporal correlations.

All available tow data for tows targetting hoki on the west coast spawning grounds between 1987 and 1993 were analysed. This area was defined as between longitudes 167°E and172°E, north of latitude 46°S and between days 150 and 335 of the year (the beginning of June to the beginning of December). For each tow, information as described in Table 1 (variables) was available. Table 1 also indicates how each variable was modelled, either as a continuous variable, with up to three parameters (cubic), or as a categorical variable, with some given number of categories.

To build a linear model of CPUE that explained as much as possible of the variation, a simple forward stepwise approach was taken. Log(catch/nautical mile) was regressed against each of a set of variables in turn. The variable that explained the most of the variability in CPUE was included in the model. This selected variable was then included in the model, and the CPUE regressed against it and each of the other predictor variables, to find the next most useful variable. This stepwise regression procedure continued until no substantial improvement came from adding an extra variable to the model (R^2 improved by less than 0.5%). Approximately 3% of the tows were reported as having zero catch. These were replaced with an arbitrary minimum catch of 1 kg before the logarithmic transform. Sensitivity tests showed that the results were not affected by the value of this arbitrary catch.

The most significant variable is a seasonal effect, which is modelled as a cubic continuous variable. As the season can be early or late in a particular year, depending on environmental factors, each season is expressed relative to the day with peak catch rates for that year. The cubic polynomial shows an increase to a peak, followed by a relatively gradual decline.

The second most important variable is nationality, modelled as a categorical variable with a category for each of the different nations with vessels in the fleet, such as Japan, Korea, and Norway. This variable could reflect either design features

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Table 1. Summary of variables in models.

Variable	Type	Description
Year	Categorical (7)	Year in which tow occurred
Season	Continuous	Day relative to peak of season for that year
End time	Continuous	Time in decimal hours at end of tow
Latitude	Continuous	Position in decimal degrees at start of tow
Depth bottom	Continuous	Depth in metres of bottom at start of tow
Depth net	Continuous	Depth in metres of ground rope of net at start of tow
Net off bottom	Continuous	Distance between ground rope and bottom at start of tow
Headline height	Continuous	Headline height of net at start of tow
Vessel nation	Categorical (6)	Country of origin of the vessel
Vessel length	Continuous	Length overall of the vessel, in metres
Vessel tonnage	Continuous	Gross tonnage of the vessel
Vessel year built	Continuous	Year the vessel was built
Vessel power	Continuous	Power of vessel engines in kilowatts
Vessel breadth	Continuous	Breadth of the vessel, in metres
Vessel draft	Continuous	Draft of the vessel, in metres
Vessel processing	Categorical (4)	Processing category of vessel
Vessel l*b*d	Continuous	Vessel length × breadth × draft in cubic metres

Note: For category-type variables, the number of categories is given in parentheses.

in the vessels involved, fishing practices, or degrees of fishing skill.

The third most important variable is the time of day that the tow ended. Again, this variable is modelled with a continuous third order polynomial, which increases through the day, peaks at about 15:00, and declines into the evening. This is consistent with fishers' perception of daily changes in catch rates in the fishery.

The fourth most important variable is the year effect. This shows a systematic change in CPUE between years that cannot be explained by changes in the distribution of fishing effort across the fishing season, changes in the composition of the fishing fleet, or other factors, and is therefore interpreted as a change in abundance of the stock.

Vessel size and the height of the net are minor variables, with the catching power of the vessel increasing with both size of vessel and size of net. Another minor variable models a trend in catch rates with latitude, with good catching areas in both the north and the south of the region. Again, these variables are modelled with cubic polynomials.

The final model can be summarized in the notation of Chambers and Hastie (1992) as follows:

$$\begin{aligned}\log(\text{catch/nautical mile}) \sim\ & \text{season} + \text{season}^2 + \text{season}^3 + \\ & \text{factor(nation)} + \\ & \text{time} + \text{time}^2 + \text{time}^3 + \\ & \text{factor(year)} + \\ & \text{length} + \text{length}^2 + \text{length}^3 + \\ & \text{latitude} + \text{latitude}^2 + \text{latitude}^3 + \\ & \text{headline} + \text{headline}^2 + \text{headline}^3\end{aligned}$$

With seven direct effect variables in the model the multiple R^2 is 23%, with 47 305 degrees of freedom. It is not surprising that only 23% of the variability can be explained using these crude variables, as even the best fishers have fluctuating catch rates within short periods. The residuals from this model are the data for the correlation studies.

Spatial and temporal correlations of model residuals

Spatial correlations

If CPUE is an index of density at a local level, then once the large-scale sources of variability are removed local patchiness in fish density might show up as structure in the residuals. We might expect that two tows that are close together would be sampling the same patch of fish, and hence would have similar residual CPUE values. Correlation between the residual CPUE values was investigated as a function of separation of the tows.

For all tows done on the same day, the closest distance between each pair of tows was calculated geometrically. A new data set was created consisting of the distance between the two tows and the two residual values from the linear model (corresponding to each of the two tows). The Spearman rank-order correlation coefficient r_s (this is the correlation coefficient of the ranks of the CPUE residuals) was calculated for the set of all pairs of tows that came within 1 nautical mile. This gives the correlation at distances up to 1 nautical mile. Similarly the correlation coefficient was calculated for sets of pairs that came within 1–2 nautical miles, 2–3 nautical miles, and so on.

The significance of the correlation coefficient was taken as

$$(1) \qquad t = r_s\,((N-2)/(1-r_s^2))^{1/2}$$

which for large N is distributed as Student's t on $N-2$ degrees of freedom (Kendall 1948).

Calculating the closest distance between a pair of tows requires some slightly complicated geometrical calculations. Approximating the earth as a sphere introduces some error, but since the distances of interest are only up to 20 nautical miles, about a third of a degree, this error is negligible. The tow was assumed to traverse a straight line between the start and end positions. This assumption may be invalid, so sensitivity to the assumption is tested later.

All positions were transformed under the Mercator projection into a coordinate system measured in radians:

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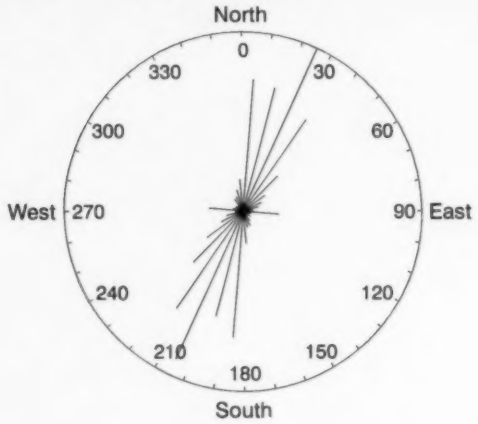
Fig. 2. Distribution of frequency of different overall courses between start and end positions of tows.



$$x = \text{longitude} \times (\pi/180)$$

$$y = \log(\tan(\pi/4 + \text{latitude} \times (\pi/360)))$$

Distances in this coordinate system were converted to distances in nautical miles as

$$\text{distance} = d \times \cos(M \times \pi/180) \times R/1.852$$

where M is the median of the latitude values in the data set, R is the radius of the earth in kilometres, and 1.852 is the conversion factor from kilometres to nautical miles.

To determine the closest distance between two tows, three different configurations were identified: (*i*) where the tows intersect, (*ii*) where the tows approach one another so that the end of one tow is close to part of the other tow (but not an end), and (*iii*) where the tows approach one another so that the end of one tow is close to the end of the other. Details of the calculations in each of these cases are available from the author.

The shortest of all these distances was the closest distance between the two tows. Note that it is not possible for two points on two different line segments, neither of which are at the end of the line segments, to be the closest points between the line segments, unless the line segments intersect.

The measure of the distance between two tows is defined here as the closest distance that the two tows come, assuming a straight path between the start and end position of each tow. Other choices could have been made, such as the distance between the start positions, the distance between the middle points, and so on. However, in any of these options, it is possible to have tows that overlap at a point or over part of the length of the tows that are measured as being a large distance apart. For example, if the measure was the distance between the start positions of the tows, then two tows that overlapped completely, but with start and finish positions swapped, would be measured as being a full tow length apart, but would be sampling the same water. In this case there could be spurious correlations at extended distances.

To eliminate this possibility, the work was carried out using the closest distance between two tows as the measure. In this case we might expect to underestimate the extent of the correlations.

Tows separated by distances in different directions

To determine whether there was any angular component to the correlation structure, the angle between the two closest points on each pair of tows was determined. The correlation structure of pairs of tows that were separated along a north–south path was then calculated, and similarly the correlation structure of pairs that were separated along an east–west path. Angles are calculated from the most northern to the most southern tow. The north–south pairs were at angles 135–225° where 0° is north. The east–west pairs were at angles either approximately east (90–135°) or approximately west (225–270°). More tows were separated along a north–south path than along an east–west path, with many about 195 or 115°.

Possibly because of the orientation of the depth contours, the distribution of courses that different tows take from start to finish position is not uniform. As shown in Fig. 2 more tows are oriented on a course about 25 or 205° than are oriented on other courses. Therefore, the angular component calculation was repeated using this NNE–SSW axis, hence tows are separated by a course either along the 25° line or at right angles to it. The pairs along the tow direction had an angle separating them of 160–250°; the pairs across the tow direction had an angle separating them of 90–160° or 250–270°.

Temporal correlations

To determine whether the spatial correlations are associated with permanent features in the environment, it is also necessary to measure the persistence of the correlations as a function of time.

Using a 10 nautical mile square grid, aligned to the longitude line 167°E and the latitude line 39.6°S, each tow was allocated to a square on the basis of its start position. For each square, the separation in days between each pair of tows in the square was calculated to create a new data set consisting of a time separation and the two residual values from the linear model. Again the rank correlation coefficient was calculated, for each separation, at 0 days (tows on same day), 1 day, 2 days, etc.

Simulation studies of the correlation structure

To determine whether spatial correlations can be generated by patches of fish measured with a fishing trawl that is comparable in length to the size of the patch (and from which we only obtain a mean density estimate), simulation studies were carried out to try to replicate the spatial correlation structure using a simple model of fish distribution. The simulation experiments involved generating square patches of fish at random (overlapping) locations within a two-dimensional matrix. The patches were located with centres at random x, y positions within the matrix such that they did not intersect the edge of the matrix. The matrix is large compared with the size of the patches, so edge effects are likely to be small. Each point in the matrix was either within, or not within, an area of fish, which was of uniform density. The matrix represented a square sea of side 500 nautical miles, within which a number of square patches of side 10 nautical miles were generated at random

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positions. The average density within the matrix is equal to the sum of the density at all points in the matrix divided by the area in square nautical miles.

Line segments were randomly located over the patches in random directions to simulate trawl shots. The line segments began at random positions within the matrix and traversed a given distance in a random direction. Line segments were permitted to leave the area of sea in which there were patches of fish. A mean density estimate was obtained by averaging the density in the locations crossed by the line. This was done by dividing the line segment into 100 points (independent of the length of the tow), and finding the density in the matrix at the location of each of the points. This mean density estimate represents the catch rate of the trawl shot.

Simulation results

In the first simulation experiment, a matrix was generated that represented a square sea of side 500 nautical miles, within which 1000 square patches of side 10 nautical miles were generated. The average density within the matrix was 0.325 per square nautical mile (patches that overlap are not counted twice). Lines of length 10 nautical miles were then placed over the patches in random directions to simulate trawl shots. A mean density estimate was obtained by averaging the density in the locations crossed by the line. Correlations were then calculated as described in the previous section. As shown in Fig. 3 a correlation structure that extends out to about 10 nautical miles is generated using this method on this simple model of the fish distribution. The extent of the correlation is similar to the size of the patches.

A matrix in which the patches were of side 20 nautical miles was then generated. This matrix had 250 square patches of side 20 nautical miles but a similar average density to the 10 nautical mile patch matrix at 0.328 per square nautical mile. As seen in Fig. 3 the correlation again persists approximately to the true known size of the patches.

Figure 4 shows the effect of the length of the tow on the extent of the correlation. The correlation is smaller when the tows are longer, but the main factor affecting the extent of the correlation is the size of the patch.

The real data set has a distribution of different tow lengths. As shown in Fig. 5 the distribution in the 1993 data set was skewed with a long tail to the right. Most tows were between 5 and 20 nautical miles. Further simulations were carried out in which the tow lengths were chosen randomly with replacement from the distribution of tow lengths in the real data set. The persistence of the correlation using this tow length distribution is again approximately the same as the patch size, for both the 10 nautical mile and the 20 nautical mile patch cases.

Another simulation investigated the effect of adding noise to the measurement of the catch rate in each trawl shot. Instead of the true mean density for each shot, a value for the catch rate was selected from a normal distribution with mean value at the true mean density for the shot and standard deviation 20 or 40% of that value. This decreases the significance of the spatial correlation slightly, but does not tend to decrease the extent of the spatial correlation.

A matrix in which the patches were of side 10 nautical miles but in which there were twice as many patches was also generated. This will affect the area of empty space in the matrix as well as the interpatch distances. The matrix had 2000 square patches of side 10 nautical miles and an average density of 0.548 per square nautical mile. The extent of the correlation structure was not affected by the change in density.

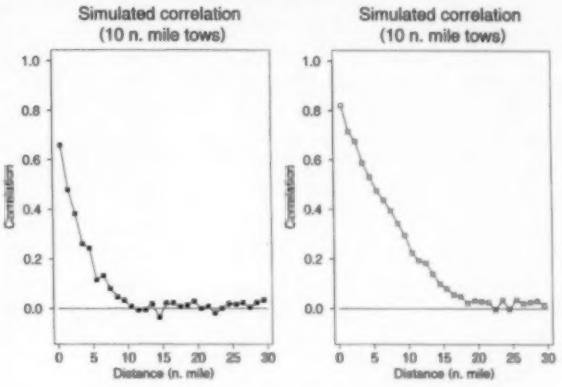

Fig. 3. Spatial correlations from a simulation with square patches of side 10 nautical miles (solid squares) or 20 nautical miles (open squares) measured with tows of length 10 nautical miles.

These simulations show that this method is capable of detecting patchiness in density distributions, even when the length of a tow is longer than the size of a patch. The method correctly estimates the size of the patch and is relatively insensitive to the length of the tows. Therefore, it is useful to apply this method to real CPUE data.

Application to the hoki fishery data

Spatial correlations

The Spearman rank-order correlation coefficient was calculated of all pairs of tows in the 1993 season that came within 1 nautical mile, 1–2 nautical miles, 2–3 nautical miles, and so on. The correlation coefficients plotted as a function of distance are shown in Fig. 6. Even at small distances the correlations are not large, only 17% for pairs 0–1 nautical mile apart. However, as this correlation coefficient was compiled with 58 904 pairs of CPUE residuals, this is a statistically significant result. The t value for this correlation is 42.3. Figure 6 also shows the t values of the correlation coefficients as a function of separation distance. A horizontal line across the plot shows that the correlations are significant at the 1% level (a t value of 2.576) for distances less than 11 nautical miles. As all separations have at least 200 pairs, the significance level at all separations is approximately the same.

Thus, tows closer than 11 nautical miles have significantly correlated catch rates. This suggests the existence of spatial structure in the fish distribution on this scale. There is also some indication that tows within 20–40 nautical miles of each other have slightly negatively correlated catch rates.

Temporal correlations

The next question, then, is if there really are patches of different densities of fish, are they related to permanent features, such as bottom contours or canyons, or to something biological or environmental? The Spearman rank-order correlation coefficient was calculated for all pairs of tows that started within

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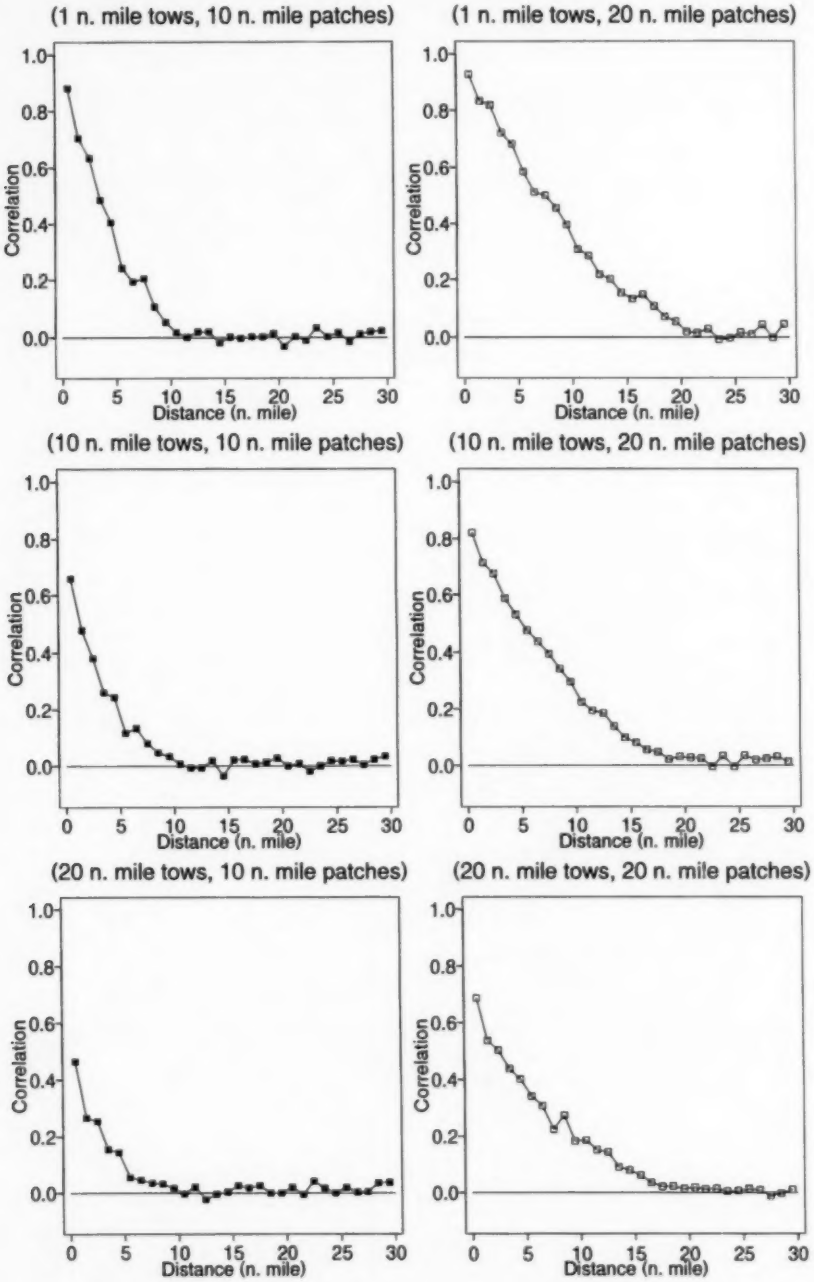
Fig. 4. Spatial correlations from a simulation with square patches of side 10 or 20 nautical miles measured with tows of length 1, 10, or 20 nautical miles.



the same 10 nautical mile square, but were separated by 0 days, 1 day, 2 days, and so on. The plot of the correlation coefficient and the *t* value (Fig. 7) show that the correlation declines rapidly, so that after 14 days there is no significant correlation between the catch rates of two tows in the same 10 nautical mile square.

The assumption of straight tows

One assumption made in this work is that the tows were largely straight between the start and end position of the tows, as entered on the fisher's form. In fact, descriptive records by scientific observers posted on some of the vessels suggested that of the (not necessarily random) sample of tows observed

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Fig. 5. Distribution of lengths of tow in a real data set (1993) and in a simulated data set.

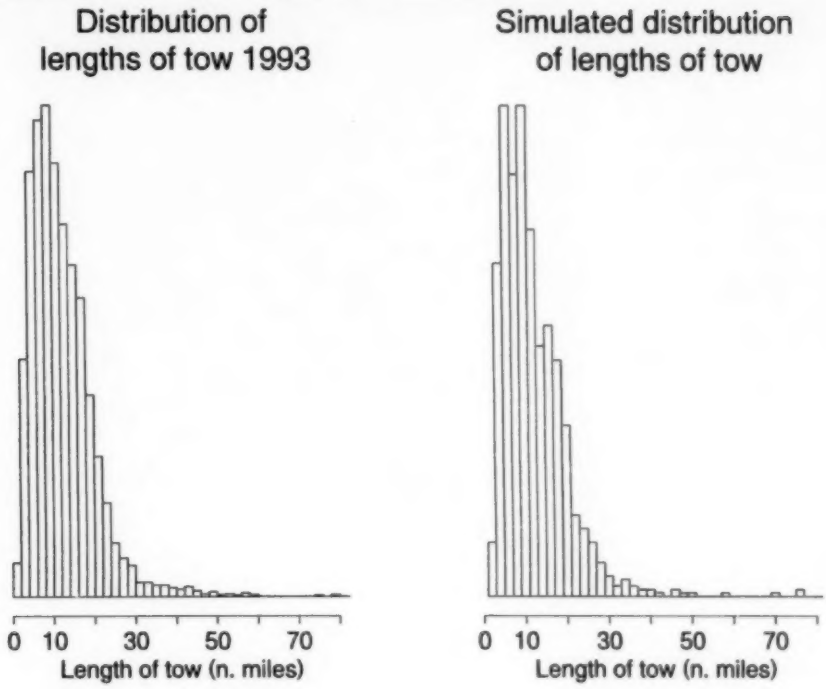
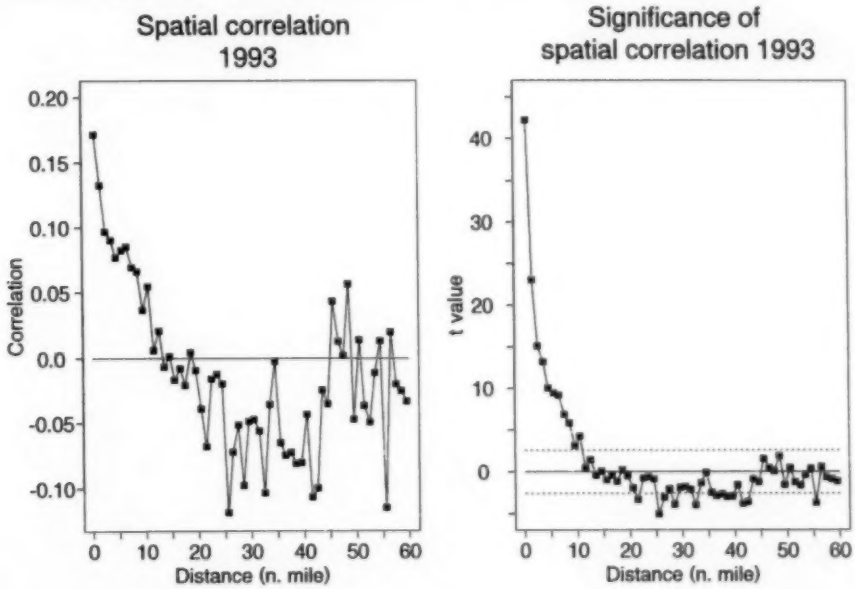

Distribution of lengths of tow 1993
0 10 30 50 70
Length of tow (n. miles)
Simulated distribution of lengths of tow
0 10 30 50 70
Length of tow (n. miles)

Fig. 6. Spatial correlations and significance of spatial correlations in residuals from linear model of CPUE, 1993.



in the 1993 season, 73% were straight, and of the rest, most involved a single U turn, coming back on the same path.

Another way of checking whether a tow is straight is to compare the displacement between the start and end positions of the tow with the distance that a vessel could travel given the speed of the vessel and the start and end times. As seen in Fig. 8 most tows went directly from start to end position (within the error associated with the start and end positions,

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Fig. 7. Temporal correlations and significance of temporal correlations in residuals from a linear model of CPUE, 1993.

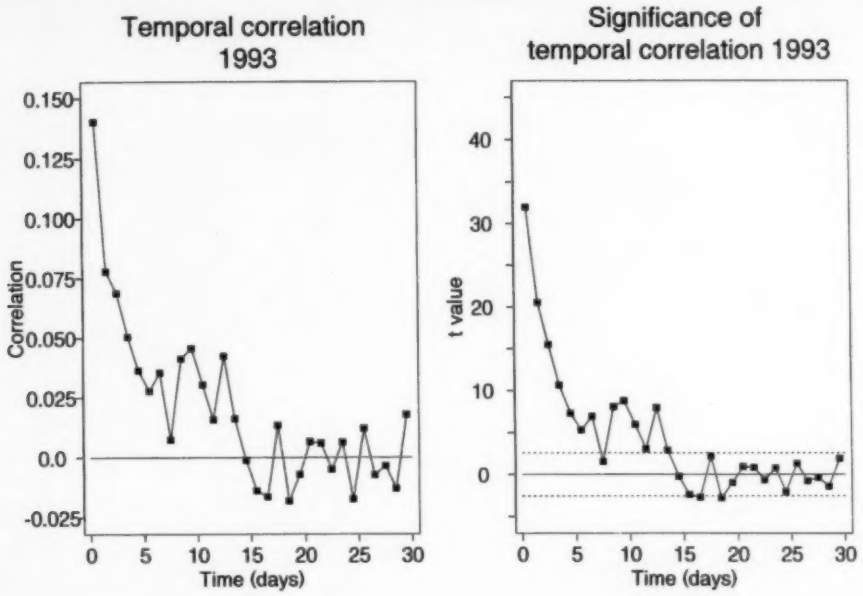
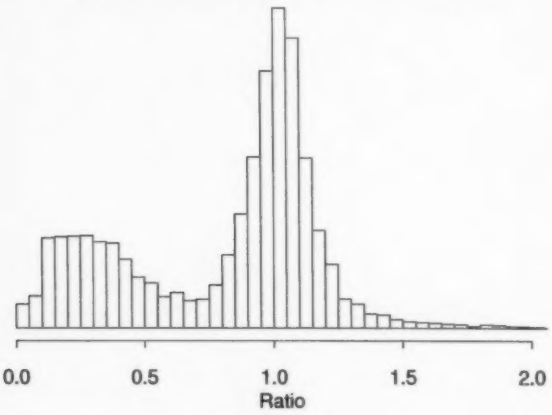


Fig. 8. Ratio of the distance travelled between start and end positions of a tow to the product of speed and duration of tow (the maximum possible distance travelled if the tow was straight).



with the start and end times, and particularly with the speed of the tow). However, there is a group of tows that achieved less than half the displacement possible. There is also a long tail of tows that apparently travelled much longer distances than was possible in the time. These are clearly reporting errors.

The analysis was repeated using only those tows in which the displacement matched the distance travelled within 50%. The magnitude of the correlations was slightly increased, but because the number of tows involved was decreased, the significance levels were slightly decreased. The scale of correlation was not changed, however.

Spatial correlation owing to vessel effects

Apart from correlations in the distribution of fish, one possible cause of the observed spatial autocorrelation is spatial correlation owing to vessel effects. A vessel is likely to do a series of closely separated tows on the same day. If catch rates of individual vessels are significantly correlated (other than as measured by the vessel size effects already included in the model), then this could cause an apparent autocorrelation of catch rates in nearby tows done by the same vessel.

The analysis was therefore repeated with all pairs of tows done by the same vessel on the same day removed from the data set of pairs of tows. The correlation pattern did not change.

Correlations in other seasons

The spatial correlation calculations were repeated for each of the years 1990, 1991, and 1992. Before 1990 fishers were not required to report the end position of the tows, so while the linear model (which uses only the start positions of tows) could use data from this period, the tow separation could not be calculated. However, in each of the years 1990–1993 there is a similar correlation pattern that extends out to at least 11 nautical miles (Fig. 9). In 1990 and 1991 the extent of significant correlation is somewhat greater than in 1992 and 1993 at 17 and 16 nautical miles, respectively, but the correlation is only just significant between 10 and 15 nautical miles, so this difference may not be real. In 1991 in particular there appears to be more correlation between 20 and 40 nautical miles, but these correlations are only barely significant.

Tows separated by distances in different directions

To determine whether there was any angular component to the correlation structure, the angle between the two closest points

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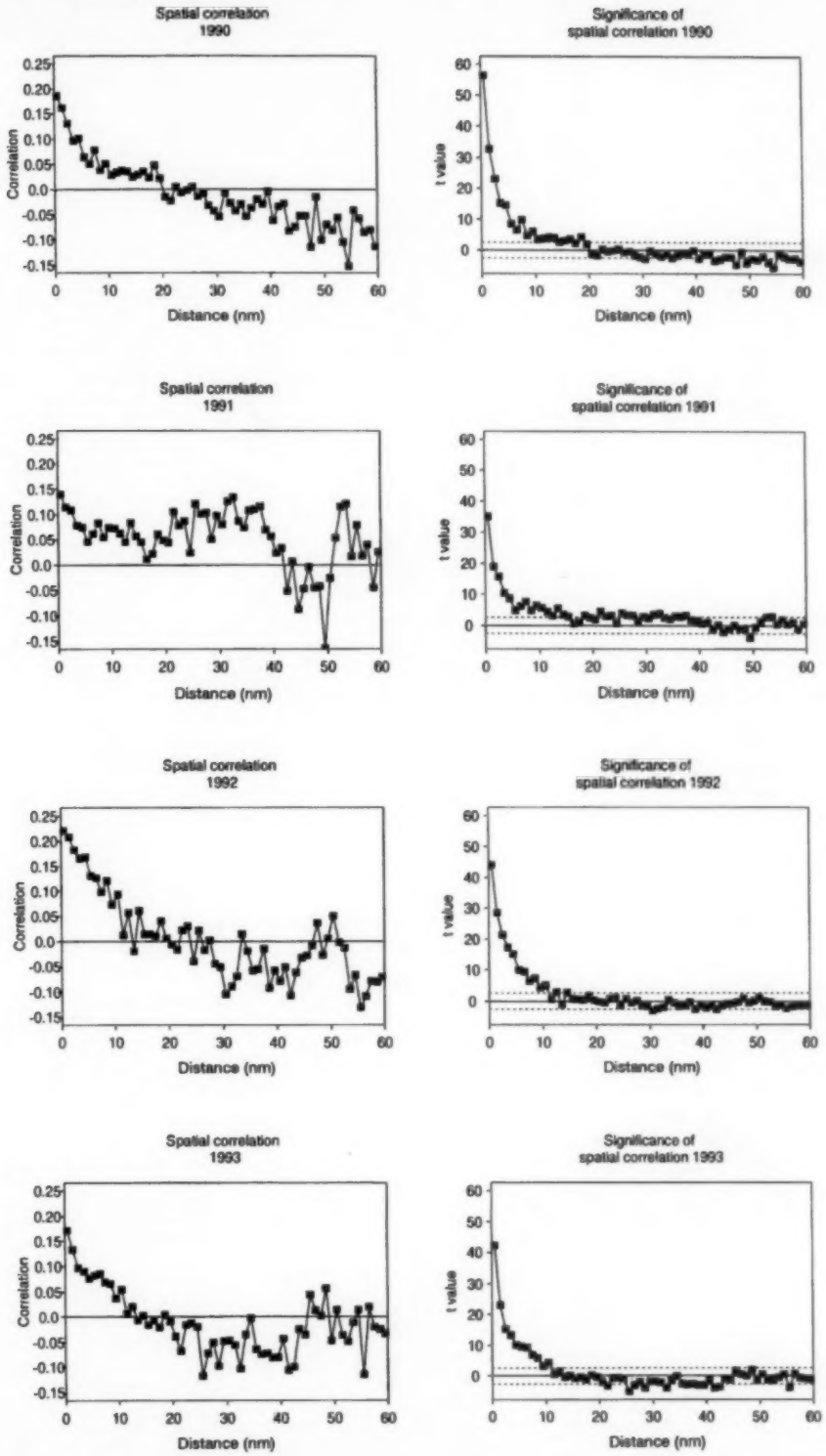
Fig. 9. Correlation structure in 1990, 1991, 1992, and 1993.



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on each pair of tows was determined. The extent of statistically significant correlation was found to be similar in both the north–south and east–west directions and along the most frequent tow direction and across it.

Discussion

This new approach to the study of catch effort data has uncovered interesting spatial and temporal structure in CPUE data by examining the residuals from a linear model that has removed as much as possible of the variability owing to vessel differences and seasonal or time of day effects. The spatial structure is on the scale of 11 nautical miles, which is of the same order, 7–30 nautical miles, as the correlation structures found by other researchers studying trawl survey or acoustic data in other fisheries (Sullivan 1991; Simard 1992; Polacheck and Volstad 1993; Petitgas 1993). If the correlation structure reflects patchiness in the distribution of fish, then the patches apparently persist for less than a fortnight, since this is the maximum temporal extent of the correlations of nearby tows. The patches therefore do not appear to be related to permanent features in the environment or the bathymetry.

Langley (1993) suggested that individual female hoki spawn several times during a 20- to 27-day period that they are resident on the spawning grounds. The 14-day time scale found in this work is less than this 20- to 27-day residence time, suggesting that an individual fish may join several aggregations during its stay on the spawning grounds.

Although the calculations were done assuming that all tows are straight, it does not seem that the extent of the correlations is due to the 30% of tows that are not straight, nor does it seem that the correlation is due to individual vessel effects not removed by the linear model.

Since the vessels clearly have a dominant tow direction, it might have been expected that this reflected an angular component in the fish distribution. One might have expected, for example, that if the fish were distributed in long cylinders along the contours, then the vessels would maximize their catch rates by following the contours. Such an angular component was not observed in the correlation structure. It may be that the distribution of tow directions of the vessels is influenced by the management measure that prohibits most of the vessels in the fleet (those over 43 m in length) from fishing within 25 nautical miles of the coast. In addition, the tendency of vessels to fish along particular tracks is self-regulating, as it becomes difficult for vessels to choose another track without causing collisions or gear entangling. Thus, it may be that vessels follow this predominant tow direction for reasons other than the distribution of the fish. However, it may also be that the symmetrical patches of fish are themselves distributed unevenly along the contours, so that in fishing along these lines, the vessels are maximizing the number of such patches that they are likely to encounter.

The true distribution of fish may be much more complex than the models in the simulations. The simulations show, however, not only that this method is capable of detecting patchiness in density distributions, even when the length of a tow is longer than the size of a patch, but also that the method correctly estimates the size of the patch and is relatively insensitive to the length of the tows. The chosen measure of the distance between tows (the closest distance between the tows) did not cause an underestimation of the size of the patches.

As well as being of scientific interest, the patchiness in the fish distribution has a number of implications for the use of catch and effort data to monitor stock size. Firstly, the existence of such correlations undermines one of the assumptions on which the linear regression model is built, namely that the data from each reported tow are independent. In fact, all tows that come within approximately 11 nautical miles of another tow will contribute slightly less information than the model assumes. Thus, the standard errors will be underestimates of the true uncertainty.

Secondly, it is important to investigate movements of vessels on the spatial and temporal scales suggested by the correlations. Any successful targetting strategy on the part of vessels must take into account the distribution of the fish. It may be important to understand the strategies that vessels are using to find fish, and in particular, variations or changes in these strategies between vessels or over a period of time, since otherwise changes in the catch rates may be confounded with changes in strategy, and this will lead to an incorrect interpretation of catch rates as an index of abundance.

Thirdly, an interaction between the patch structure and the vessel strategy may affect catch rates in a particular year, as the ability of the vessels to target patches may have an important influence on overall catch rates. In years when patch size is unusual, or when patches are less distinct, catch rates may drop as vessels find it difficult to target fish or find that their traditional strategies are less successful.

Finally, if vessels are successful in targetting the best patches of fish then a simple linear model of catch effort data will tend to index only the density within patches, rather than the true abundance. In fact, changes in the size of the patches may be important indications of changes in abundance that cannot be monitored by the catch rates themselves. It may be more important to monitor the size of patches, using the correlation structure, than to monitor the average catch rates. For all these reasons, these spatial and temporal correlations may prove fundamental to understanding the relationship between catch rates and abundance in this fishery.

Acknowledgments

I thank Kevin Sullivan, Elizabeth Bradford, and two reviewers for useful comments on earlier drafts.

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The genetic stock structure of common snook (*Centropomus undecimalis*)

Michael D. Tringali and Theresa M. Bert

Abstract: Protein electrophoresis and mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLP) analysis were used to analyze the population structure and identify fishery stocks of common snook (*Centropomus undecimalis*) in the western Atlantic Ocean. For the species, measures of variability in both allozymes and mtDNA RFLPs were low compared with those for fishes with similar life histories, including congeners. In Florida, common snook are sufficiently divergent in mtDNA sequence between the Atlantic and Gulf of Mexico that these distinct stocks should be treated as separate management units. Particular RFLPs that were absent from Caribbean snook were prevalent in and endemic to Florida snook, indicating that genetic exchange between populations from these regions is also low. Although we observed no differences in the distribution of mtDNA haplotypes among Caribbean island samples, F_{ST} values were comparatively high, indicating that allozyme heterozygosity among these populations was substructured. As with many marine fishes, stocks of common snook were identified principally by differences in genotypic frequencies and in levels of genetic diversity rather than by strict geographic groupings of mtDNA haplotypes or of fixed allelic differences.

Résumé : Les auteurs ont eu recours à l'électrophorèse des protéines et à l'analyse du polymorphisme de la longueur des fragments de restriction (RFLP) de l'ADN mitochondrial (ADNmt) pour analyser la structure des populations du crossie blanc (*Centropomus undecimalis*) et distinguer les différents stocks dans l'océan Atlantique Ouest. À l'échelle spécifique, la variabilité associée aux allozymes et au polymorphisme des fragments de restriction de l'ADNmt était faible comparativement à celle observée chez des espèces présentant des cycles biologiques similaires, y compris des espèces appartenant au même genre. En Floride, les séquences d'ADNmt diffèrent suffisamment entre les stocks de l'Atlantique et du golf du Mexique pour qu'on puisse considérer ces stocks comme des unités de gestion distinctes. Des fragments de restriction particuliers absents chez des crossies blancs des Antilles ont été observés à l'état endémique chez des crossies de la Floride, ce qui indique que les échanges génétiques entre les populations de ces régions sont également peu fréquents. Bien que les auteurs n'aient observé aucune différence entre la distribution des haplotypes d'ADNmt parmi les échantillons provenant des Antilles, les valeurs F_{ST} étaient en comparaison élevées, ce qui indique que l'hétérozygotie des hallozymes est substructurée chez ces populations. Comme chez de nombreuses espèces de poissons marins, les stocks de crossie blanc se distinguent avant tout par des différences dans les fréquences génotypiques et par le degré de diversité génétique, plutôt que par des regroupements géographiques stricts d'haplotypes d'ADNmt ou de différences alléliques fixes.
[Traduit par la Rédaction]

Introduction

Common snook, *Centropomus undecimalis*, are important high-level carnivores in tropical and subtropical coastal marine waters, estuaries, and rivers. The species ranges in the western Atlantic Ocean and Gulf of Mexico from central Florida and southeastern Texas southward to Rio de Janeiro, Brazil, and those Caribbean and Antillean islands having permanent freshwater sources joining the sea (Rivas 1986). Common snook are euryhaline and semicatadromous. Adults are generally found in rivers and inland waterways during winter (Taylor et al. 1993*b*), but during summer they move into saline and estuarine waters to spawn; spawning occurs at the mouths of inlets and along beaches during spring low tides. Rising tides facilitate the movement of eggs toward freshwater environments, habitats that larvae occupy. Juveniles inhabit both freshwater and brackish-water areas (Gilmore et al. 1983; Peters 1993). Like the confamilial barramundi (*Lates calcarifer*), common snook are protandrous hermaphrodites (R. Taylor and H. Grier, Florida Department of Environmental Protection, St. Petersburg, Fla., personal communication); individuals mature and spawn first as males before changing sex at older ages.

Common snook are an important food resource for Central and South American countries (Lemos et al. 1980) and a valued gamefish in Florida (Marshall 1958). Managing the fishery for common snook in Florida has been difficult historically because of fluctuations in population size and because of ambiguity concerning regional differences in population biology (Bruger and Haddad 1986; Taylor et al. 1993*a*, 1993*b*). The temporal stability of common snook abundance in Florida is greatly influenced by environmental factors (Storey and Grudger 1936; Gilmore et al. 1978), particularly water temperature, which in winter (Collier and Hedgpeth 1950; Hoese and Moore 1977) can descend below the lower lethal temperature range for snook (8–14°C (Shafland and Foote 1983; Howells et al. 1990)). Several components of the population biology of snook from the Atlantic Ocean coastal waters of Florida differ notably from those of snook from the Gulf of Mexico coastal waters of Florida. Compared with snook from

Received October 13, 1994. Accepted December 7, 1995.
J12603

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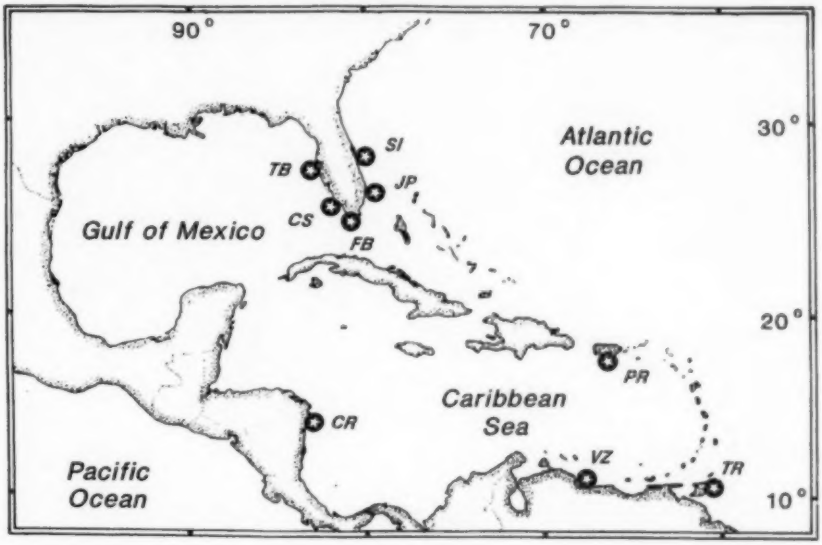
Fig. 1. Collecting locations for common snook, *Centropomus undecimalis*. Florida Atlantic: SI, Sebastian Inlet; JP, Jupiter – Palm Beach. Florida Gulf of Mexico: FB, East Cape Sable, Florida Bay; CS, Collier – Seminole State Park; TB, Tampa Bay. Caribbean: CR, Boca Tortuguera – Rio Colorado, Costa Rica; VZ, Rio Chico, Venezuela; TR, Port-o-Spain, Trinidad; PR, Anasco River, Puerto Rico.



the Florida Gulf, snook from the Florida Atlantic have a larger size at age, older age at maturity, greater longevity of females, and lower natural mortality (Taylor et al. 1993*a*).

The importance of common snook to the recreational fishing industry in Florida and concern about the significance of the regional differences in common snook population abundance and biology stimulated interest in understanding the geographic structure of common snook stocks. Molecular population genetics is a well-established and necessary tool used in the identification of fish stocks for management purposes (Utter 1991). Significant, temporally persistent, genetic differences between animals from different locations indicate that a species is subdivided into discrete, reproductively isolated units (non-interbreeding groups) (Ovenden 1990). Once independent stocks are identified, their population dynamics, especially recruitment and mortality, may be studied separately and programs established to manage each of those populations independently (Allendorf et al. 1987; Pope 1980).

To address fishery management concerns and to obtain a perspective on the general genetic stock structure of common snook, we used protein electrophoresis to examine variation in allozymes and restriction fragment length polymorphism (RFLP) analysis to examine variation in mitochondrial DNA (mtDNA) in fish from a large portion of the species' range. Protein electrophoresis provides data that can be used to reveal the variation in biparentally inherited nuclear genes coding for metabolic enzymes, whereas mtDNA RFLP analysis reveals the variation in the more rapidly evolving, uniparentally inherited, extranuclear mtDNA (Brown et al. 1979). Patterns of genetic variation revealed by the two techniques are interpretable in a geographical, ecological, or evolutionary context and, for snook, can be integrated to provide information on migration (e.g., Avise 1986; Ovenden et al. 1992) and genetic stock structure (e.g., Gold et al. 1993).

Materials and methods

Sample collection

Common snook were collected by hook-and-line fishing, trammel and cast netting, and electroshocking from nearshore, estuarine, or riverine waters off the following coasts: Florida Atlantic (two locations; high-salinity inlets), Florida Gulf of Mexico (three locations; high-salinity estuaries or bays), Costa Rica (two adjoining riverine locations; samples were pooled for analysis), Venezuela (brackish lagoon), and Puerto Rico (brackish lagoon). Specimens caught in fresh water were also obtained from a fish market in Trinidad (Fig. 1). All specimens were collected between February 1990 and July 1993. Somatic muscle, heart, liver, and gonadal tissue were taken for protein electrophoresis and mtDNA RFLP analysis. In most cases, sex and fork length were recorded for each individual prior to dissection; fork lengths averaged 454 mm (range 102–683 mm). Samples were frozen in liquid nitrogen or on dry ice and stored at –80°C in the laboratory until processing.

Protein electrophoresis

Protein electrophoretic procedures were modified from Bert (1986). Initially, the four tissue types from 10 common snook from Florida were screened for detectable activity of many enzymes; activity was best detected in muscle and liver tissues. Twenty-one proteins were resolved, representing 31 putative genetic loci. For each protein resolved, tissue types and buffer systems used, number of putative genetic loci identified, and enzyme structures are listed in the Appendix. We assayed 241 individuals for the 31 loci. For multilocus enzyme systems, loci were enumerated from the anodal end of the gel toward the cathodal end.

We used the BIOSYS statistical program (Swofford and Selander 1981) to examine genotype frequencies of each locus for conformation to Hardy–Weinberg equilibrium expectations (χ^2 goodness of fit test with and without pooling of rare alleles and corrected using the sequential Bonferroni technique (Rice 1989)), average heterozygosity per individual (H_o), the percentage of loci polymorphic in a sample (P_{95}, where a locus is defined as polymorphic if the maximum

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frequency of the most common allele in any sample does not exceed 95%), genetic distances between samples (Nei's unbiased genetic distance; Rogers' modified distance (Wright 1978)), and phenetic clustering of samples (UPGMA applied to Nei's distances; distance Wagner procedure applied to Rogers' modified distances). To test for the independence of allele frequencies from sample location, we applied the $R \times C$ G test of independence (Sokal and Rohlf 1981) to each locus defined as polymorphic at the P_{95} level. Loci at which allele frequencies were significantly heterogeneous were further evaluated using the simultaneous test procedure for frequencies (STP; Sokal and Rohlf 1981) to locate the source(s) of the significance.

Structuring of heterozygosity within common snook was examined using Weir and Cockerham's (1984) θ (F_{ST}) measure, including a jack-knifed estimation of standard error. The relationship of θ to geographic distance was analyzed as in McMillen-Jackson et al. (1994), except that values of θ, rather than estimated gene flow values, were plotted. Beginning with a reference sample, θ was calculated for a sample set composed of a reference sample and its nearest coastal or island clockwise-neighboring sample. Samples were then added to the sample set one at a time, moving in a clockwise direction, and θ was estimated after each addition. The process was continued until all samples were included in the estimate of θ. Each sample, in turn, served as the reference sample, so that θ was estimated for all geographically sequential combinations of samples.

mtDNA RFLP analysis

Total cellular DNA was obtained using approximately 100–200 mg of liver or somatic muscle tissue from 163 common snook from the nine sampling sites. We surveyed 68 individuals using the following restriction enzymes that recognize six-base nucleotide sequences (six-base enzymes): *Apa*I, *Bam*HI, *Dra*I, *Eco*RV, *Hin*dIII, *Pvu*II, *Pst*I, *Sma*I, *Sst*I, *Sst*II, and *Xba*I. Sixty of these individuals were further analyzed using the following restriction enzymes that recognize four-base nucleotide sequences (four-base enzymes): *Alu*I, *Bst*NI, *Cfo*I, *Hae*III, *Hin*fI, *Mbo*I, *Msp*I, *Rsa*I, and *Sty*I. The remaining 96 individuals were assayed using only the four-base enzymes found to be polymorphic: *Alu*I, *Cfo*I, *Hae*III, *Mbo*I, *Msp*I, and *Rsa*I.

Frozen tissues were powdered in liquid nitrogen and digested at room temperature for 15 min in 1.0 mL Lifton's buffer (0.2 M sucrose, 50 mM ethylenediaminetetraacetic acid (EDTA), 100 mM Tris, pH 9.0) containing 25 µL of 20% SDS. The SDS and cellular debris were removed by precipitation in 0.15 mL of 8 M potassium acetate (pH 8.0) for 30 min at –20°C and centrifuging at 16 000 × g. The DNA was purified using the phenol–chloroform extraction procedure of Maniatis et al. (1982) except that two chloroform–isoamyl alcohol (24:1 v/v) extractions were performed. The DNA was concentrated by isopropanol precipitation, resuspended in 100 µL TE buffer (1 mM EDTA, 10 mM Tris, pH 8.0), and stored at –20°C. Restriction enzyme digests were carried out according to the manufacturer's (Boehringer Mannheim) directions using 2–5 µL of the DNA sample. The resulting DNA fragments were electrophoretically separated on agarose gels, transferred to charged nylon filters (ZetaBind; CUNO, Inc.) (Maniatis et al. 1982), and bound to the filters by baking (30 min, 120°C) or crosslinking (1230 kJ). We visualized the mtDNA fragments using procedures described in Tringali and Wilson (1993), except that a chemiluminescent reaction (Lumi-Phos 530; Boehringer Mannheim) was substituted to increase signal (band) intensity. Autoradiograph exposure times normally ranged over 1–5 min. The sizes of the mtDNA fragments were estimated graphically by comparing them with DNA molecular weight standards. Fragments smaller than 200 base pairs (bp) were not scored.

Distinct restriction fragment patterns were assigned a specific letter; A represented the most common pattern. Individual mtDNA haplotypes were considered to be the composite of the restriction fragment patterns produced by the six polymorphic four-base enzymes. Percent nucleotide divergence values, p (Nei 1987), were calculated between pairwise comparisons of unique mtDNA haplotypes and between pairwise comparisons of samples from each location using the RESTSITE computer program (version 1.2; Nei and Miller 1990); divergence estimates between sample locations were corrected for within-sample variability (Nei 1987) and bootstrap standard errors were calculated using 200 bootstrap cycles. The nucleon (haplotype) diversity index (h) was estimated for each sample, including an estimate of standard error (Nei 1987).

For analysis of population structure, mtDNA haplotype frequencies of the entire set of locations were tested for geographic heterogeneity using the χ^2 statistic and Monte Carlo bootstrap procedure (Roff and Bentzen 1989). Significant heterogeneity among locations was further evaluated using the STP. The V test (DeSalle et al. 1987) was used to test for heterogeneity of proportions of particular RFLP patterns (following arcsine square-root transformation) among regions. Population structure was also examined by constructing neighbor-joining trees from the p values estimated between samples. The genealogy of mtDNA haplotypes within the species was examined phenetically; we used UPGMA to cluster p values estimated between mtDNA haplotypes and calculated the standard errors of branch lengths using the algorithm of Nei et al. (1985).

Results

Allozyme analysis

Allele and genotype frequencies are available from the authors upon request. Overall, allozyme variability was low (Table 1). Only eight loci were polymorphic in at least one sample at the P_{95} level; but at each of these loci, the common allele usually occurred at a high frequency (≥0.90). Notably, the Trinidad sample lacked polymorphism at any locus. Only one locus deviated significantly from Hardy–Weinberg equilibrium expectations in a single population (*IDH* at Venezuela).

Of eight loci tested for geographic heterogeneity in allele frequencies among samples, only *GPI-1* showed significant heterogeneity (Table 2); the *GPI-1* allele frequencies for the Collier–Seminole sample differed significantly from those of the Trinidad sample. Nei's and Rogers' modified genetic distances computed using all loci were very low for all pairwise combinations of populations (Nei's, ≤ 0.002; Rogers' modified, ≤ 0.045), and the phenograms produced using these distances were unresolved.

The average value of θ over all loci was 0.010 ± 0.002 (mean ± SE); F_{IS} and F_{IT} were 0.018 and 0.028, respectively, indicating that the genetic variation, although relatively low, was partitioned both within and among samples. In the geographic analysis of heterozygosity, θ values for mainland populations were generally low, but nevertheless revealed population structuring (Fig. 2). The θ values for groupings that included only Florida Atlantic samples did not differ significantly from zero. However, all θ values calculated for groupings that included Florida Atlantic samples and other samples, except the Florida Bay sample, were significant. Similarly, nearly all θ values for groupings that included Florida Gulf and Caribbean mainland samples did not differ from zero. However, again with the exception of the Florida Bay sample, θ values for all groupings that included Florida Gulf and (or) Caribbean mainland samples and Florida Atlantic or Caribbean island samples did differ significantly from zero. Finally, θ values for all groupings that included the two Caribbean island samples differed significantly from zero. Overall, the sequential θ calculations support the following ideas: (*i*) gene flow between Florida Atlantic snook and those from other

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Table 1. Measures of genetic variability of common snook, *Centropomus undecimalis*, from different locations.

	Protein electrophoresis			mtDNA		
Location	n	P_{95}	$\bar{H}_o$	n	h	p
SI	20	13	0.022±0.010	19	0.83±0.04	0.28±0.09[a]
JP	29	3	0.024±0.009	24	0.82±0.03	0.16±0.05
FB	16	7	0.023±0.010	16	0.50±0.09	0.06±0.04
CS	62	10	0.032±0.012	20	0.63±0.08	0.08±0.03
TB	60	7	0.025±0.011	21	0.33±0.09	0.08±0.02
CR	22	19	0.031±0.013	19	0.73±0.06	0.09±0.04
VZ	12	13	0.032±0.014	16	0.63±0.10	0.08±0.02
TR	8	0	0.000	8	0.00	0.00
PR	12	10	0.020±0.011	12	0.54±0.11	0.09±0.05

Note: Measures of variability were determined from protein electrophoretic data (P_{95}, percentage of loci polymorphic in a sample (subscript denotes maximum frequency of the most common allele); $\bar{H}_o$, average percentage of loci heterozygous in an individual) and by mitochondrial DNA restriction fragment length polymorphism analysis (h, nucleon diversity index; p, percent nucleotide sequence divergence). Values are given as means ± standard errors. n, number of individuals sampled. Abbreviations for sampling locations are identified in Fig. 1.

[a]Value includes the highly deviate mtDNA haplotype 9; value excluding that haplotype is 0.25 ± 0.05.

Table 2. Allele frequencies for the presumptive protein-coding locus *GPI-1* in the common snook, *Centropomus undecimalis*.

	Location								
GPI-1 allele	TR	JP	SI	VZ	FB	CR	TB	PR	CS
100	1.00	0.97	0.90	0.92	0.87	0.86	0.85	0.83	0.81
110	—	0.03	0.05	0.08	0.13	0.14	0.15	0.17	0.19
105	—	—	0.05	—	—	—	—	—	—
n	16	58	40	24	32	44	120	24	124
STP analysis									

Note: See Fig. 1 for definitions of location abbreviations and the Appendix for the definition of the locus abbreviation. n, number of genes sampled for each locus; —, frequency of 0.0. For the STP analysis (defined in Materials and methods), samples grouped by a continuous rule were not significantly different from each other ($P > 0.05$).

locations (including west-central Florida) is low; (*ii*) south Florida is an area of mixing of separate Florida Atlantic and Florida Gulf snook populations; (*iii*) gene flow between Florida Gulf and Caribbean mainland snook is, or once was, high; and (*iv*) Caribbean island snook populations are comparatively isolated.

mtDNA RFLP analysis

The mtDNA genome size of common snook, calculated from the averaged sums of the restriction fragments of all restriction profiles produced by the six-base enzymes, was approximately 17 700 ± 50 bp. A restriction site map of six-base enzymes was obtained using double digests and is available from the authors upon request. mtDNA size variation was putatively observed in some individuals in the RLFP patterns produced by *Cfo*I, *Bst*NI, *Hae*III, *Hin*fI, and *Mbo*I. Putative size variants were observed in all samples. Although genomic size variation due to tandem repeats in the D loop has been observed in common snook (R. Wilson, University of South Florida, St. Petersburg, Fla., personal communication), our methodology does not allow us to completely rule out other explanations for the observed differences in RFLPs. However, because the magnitude of size variation in particular individuals was consistent among those five restriction enzymes, it is unlikely that restriction sites resulting in undetectably small fragments were mistaken for size variation.

In the 60 individuals scored for all 20 six-base and four-base enzymes, a total of 205 unique fragments were detected, representing approximately 5.0% of the snook mtDNA genome. The amount of polymorphism detected by the six-base enzymes was very low. Eight of the 11 six-base enzymes detected only single restriction fragment patterns. The enzymes *Bam*HI and *Dra*I detected, in each case, only a single individual that deviated from the common pattern by a single restriction site; the enzyme *Eco*RV detected two individuals that deviated from the common pattern by single, different restriction sites. In contrast, the fragment patterns of six of the nine four-base restriction enzymes were moderately to highly polymorphic, i.e., at least 5% of the fragment patterns detected by a given enzyme varied from the common pattern. Our populational and genealogical analyses based upon the 156 snook completely assayed using these six enzymes accounted for 134 unique fragments or 3.0% of the genome.

Two of the four polymorphisms that we observed using six-base enzymes occurred in a single snook from Sebastian Inlet. Six of the nine four-base enzymes also produced variant mtDNA restriction patterns in this snook (Table 3, haplotype 9). We confirmed the species identification of this highly variant individual using protein electrophoresis and DNA sequencing of the cytochrome *b* gene for common snook and all other snook species (T. Bert, M. Tringali, and S. Seyoum, unpublished data). Although we believe haplotype 9 to be simply

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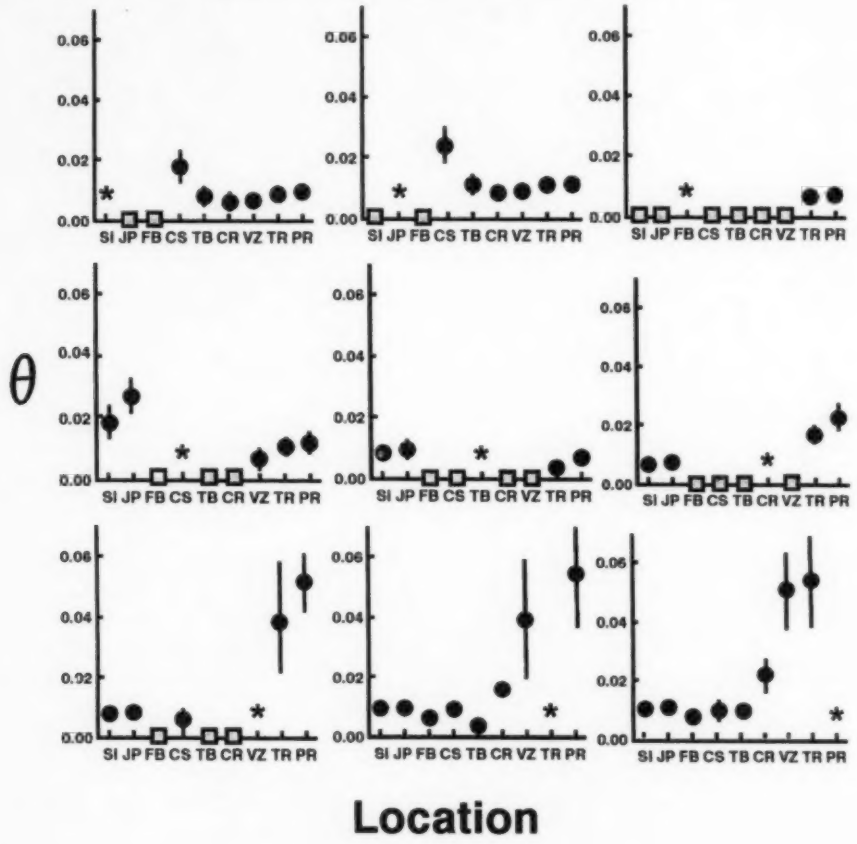
Fig. 2. Geographic structure of heterozygosity in *Centropomus undecimalis*. In each histogram, the symbol above each location represents the θ value (Weir and Cockerham 1984) estimated for samples from all locations between and including that location and the reference location (denoted by an asterisk). Squares depict estimates that did not differ significantly from zero; circles depict estimates that differed significantly from zero. Vertical lines represent standard errors of θ. Location abbreviations are defined in Fig. 1.



a relict lineage (the stochastic processes of mtDNA lineage sorting and extinction often lead to a mixture of both closely and distantly related lineages (Neigel and Avise 1986; Bernatchez and Dodson 1991)), we analyzed relevant data sets both with and without this individual.

The polymorphic four-base enzymes produced 33 unique mtDNA haplotypes (Table 3). Overall, the majority of snook (58%) were typified by haplotype 1; however, the frequency of this haplotype varied from 100% in the Trinidad sample to 32% in the Sebastian Inlet sample. Seven haplotypes (Table 3, haplotypes 3, 5, 7, 11, 13, 16, and 27) were observed in snook from multiple locations, but at relatively low frequencies. The majority of haplotypes (76%) were specific to particular locations and also occurred in low frequencies. Among samples, values of h differed markedly (range 0.00 for Trinidad to 0.83 ± 0.04 for Sebastian Inlet; Table 1). Notably, h values from the Florida Atlantic were higher than those from all other samples. Significant heterogeneity was observed among mtDNA haplotype frequencies over all populations (χ^2 = 302.4, P = 0.009). The mtDNA haplotype frequencies of the two Florida Atlantic samples differed significantly from those of the Tampa Bay and Florida Bay sample. The haplotype distribution of the Trinidad sample did not differ from that of any other sample, probably because of the small size of the Trinidad sample and because only the most common haplotype occurred in that sample.

The p values among individuals within locations ranged from 0.00% (TR sample) to 0.28 ± 0.09% (SI sample) (Table 1). Because these estimates were made from RFLPs generated using only polymorphic enzymes, they should not be used in making general comparisons regarding the amount of mtDNA variability between common snook and other species. The overall value p = 0.05% (±0.006%), calculated using the mapped restriction site data generated from the six-base restriction enzymes and samples from all locations combined, is appropriate for that purpose. Pairwise p values between samples from different locations, corrected for within-sample variability, ranged from 0.00% (in the following pairs of samples: CS vs. TB, CS vs. FB, FB vs. TB, SI vs. JP, VZ vs. TR) to 0.0162 ± 0.0083% (PR sample vs. SI sample). In the neighbor-joining cluster analysis of p, snook from the Florida Gulf were far more similar to Caribbean snook (CR, PR, VZ, TR) than

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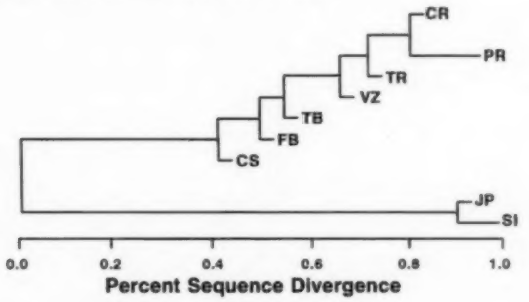
Table 3. The mtDNA haplotypes and haplotype distributions for 156 *Centropomus undecimalis* from Florida and the Caribbean.

Haplotype no.	mtDNA haplotype	Location								
		TB	CS	FB	JP	SI	CR	VZ	TR	PR
1	AAAAAA	17	12	13	9	6	9	9	8	8
2	BAAAAB	2								
3	ABAAAA	1	2		2	1				
4	AAAECA	1								
5	ACCABA				2		1			
6	ACAABB					1				
7	AAAAAB		1	2	5	3				
8	AAAABB					1				
9	AEBDDE					1				
10	ABCAAB					1				
11	AAAABA				3	2	1	1		
12	ACAAAA					1				
13	ADAAAA					2	1	1		
14	AAAAEB		1							
15	ACAABA		1							
16	AAAAAF		1					1		
17	AAABAA		1							
18	AAEAAA		1							
19	AACAAB				1					
20	ACCAAA				1					
21	AACAAA				1					
22	AAAAAC						4			
23	AGAAAA						1			
24	AFAAAA						1			
25	AAFAAA						1			
26	AAACAA							1		
27	CAAAAA							1		1
28	AADAAA							1		
29	AAAAAD							1		
30	AAAAFB			1						
31	AAAAAG			1						
32	AAAEAA									1
33	AAAAAH									2
Total		21	20	17	24	19	19	16	8	12

Note: Letter code denotes the composite restriction fragment pattern for the following enzymes: *Cfo*I, *Rsa*I, *Alu*I, *Hae*III, *Msp*I, *Mbo*I. Abbreviations for locations are given in Fig. 1.

Fig. 3. Analysis of the genetic population structure of *Centropomus undecimalis*. Neighbor-joining tree based on estimated mtDNA percent sequence divergence values (p). Location abbreviations are given in Fig. 1.



to snook from the Florida Atlantic (Fig. 3). Reanalyzing the data without haplotype 9 did not affect the relationships among locations nor the distance observed between the Florida Atlantic samples and others.

Snook mtDNA haplotypes clustered into four putative clonal groups (Fig. 4). Group A consisted of a multichotomous arrangement of haplotypes derived from the common haplotype; most branches within that group were not strongly supported. Groups B and C differed significantly from each other, although neither group could be separated from group A. Group D was represented by the single, highly divergent individual from Sebastian Inlet. Reanalyzing the data without haplotype 9 did not affect the relationships within or among the other clonal groups. The cluster analyses did not indicate strong geographic partitioning of haplotypes overall, but some phylogeographic structure was evident. Group B was dominated by haplotypes from Florida Atlantic snook, and group C was dominated by haplotypes from all five Florida samples. All "Florida" haplotypes in group C and three "Florida Atlantic"

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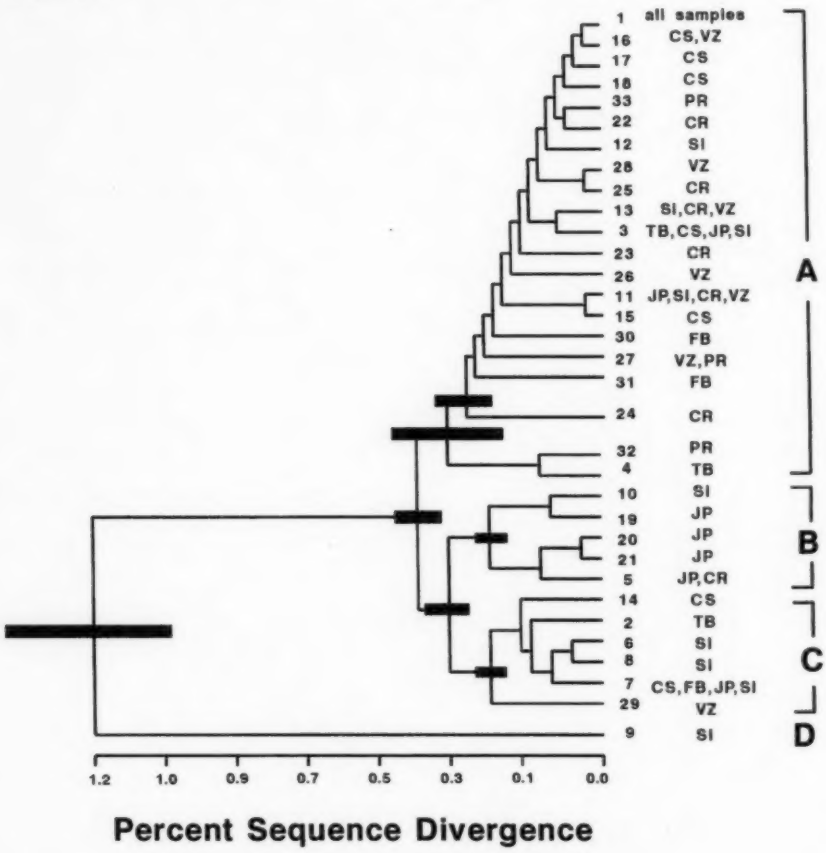
Fig. 4. Genealogical analyses of mtDNA haplotypes observed in *Centropomus undecimalis*. UPGMA phenogram based on percent nucleotide sequence divergence values. Clusters A through D represent putative clonal groups derived from the UPGMA analyses. Definitions of location abbreviations are given in Fig. 1; definitions of haplotype numbers are given in Table 3. Standard errors of the branching points were estimated as in Nei et al. (1985).



haplotypes in group B had the "B" pattern for *Mbo*I; overall, 19% of all Florida individuals had this pattern, which was absent in Caribbean snook. The frequency of occurrence of this RFLP differed significantly between the Florida and Caribbean samples ($V = 21.35$, $P < 0.001$) but not among Florida samples. Another restriction pattern (*Rsa*I-B) appeared in 7% of individuals from Florida but was absent in Caribbean snook; the frequency of this RFLP also differed between the Florida and Caribbean samples ($V = 6.19$, $P < 0.025$) but not among Florida samples.

Discussion

Genetic diversity

The level of both allozyme and mtDNA variability in common snook is low compared with that of many other marine or semi-catadromous fish species. For 106 species of marine and semi-catadromous teleosts, $\overline{H}_o$ averaged 0.055 ± 0.036, and tropical and subtropical wide-ranging species generally had the highest values (Smith and Fujio 1982). The $\overline{H}_o$ value for common snook (0.025 ± 0.011) is at the low end of the range for these fishes. Even the $\overline{H}_o$ values for the western Atlantic congeneric *Centropomus parallelus* (0.079 ± 0.030), *Centropomus pectinatus* (0.062 ± 0.023), and *Centropomus ensiferus* (0.071 ± 0.030) are markedly higher, as are $\overline{n}_a$ (average number of alleles per locus) and P_{95} values (M. Tringali and T. Bert, unpublished data). The mtDNA p value for common snook (0.05 ± 0.006%) is also among the lowest reported. Typical p values for marine fishes have ranged 5–0.5% (Ovenden 1990). Only the anadromous striped bass, *Morone saxatilis*, and marine black sea bass, *Centropristis striata*, have reported p values lower than those reported for common snook (Wirgin et al. 1989; Bowen and Avise 1990). For striped bass and black sea bass, evolutionarily recent bottleneck events were hypothesized to account for the observed paucity of genetic variability. A widespread bottleneck event could also be hypothesized for common snook (genetic bottlenecks have a greater affect upon the mtDNA genome than the nuclear genome (Birky et al. 1983)), assuming that the rate of mtDNA evolution for this species is similar to that recently observed in other fishes (Martin et al. 1992; but see Martin and Palumbi 1993).

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Geographic structure of variation

The geographic patterns of allozyme and mtDNA genetic variability we observed indicate that common snook are subdivided into genetically differentiated populations between the Atlantic and Gulf waters of Florida and between Florida and Caribbean waters. Caribbean island populations appear to be isolated as well. Florida Atlantic snook are genetically quite distinct from all others.

Substantial mtDNA divergence, both in haplotype diversity and in nucleotide sequence, is clearly evident between snook from the Florida Gulf and Florida Atlantic waters, supporting the hypothesis that the two populations are reproductively isolated. Tagging studies also indicate that gene flow between Florida Gulf snook and Florida Atlantic snook does not occur and that the two populations have diverged. Of 4100 snook tagged and recovered in the Florida Gulf and Florida Atlantic, none had migrated between the waters of the two coasts, although more than 1700 had been at large for more than a year (Volpe 1959; R. Taylor, G. Bruger, and J. Whittington, Florida Department of Environmental Protection, St. Petersburg, Fla., unpublished data). In addition to the biological differences mentioned in the Introduction between snook from the two coasts, there are marked differences in the migratory behaviors of snook inhabiting the waters of the two Florida coasts. In the Florida Atlantic, more than 40% of all snook recaptured (n = 1947) had migrated 50–350 km along the Atlantic coast, and snook consistently migrated between the two Atlantic locations we sampled. Conversely, in the Florida Gulf, more than 99.5% of the 2053 snook tagged and returned were recaptured in their home estuaries (<10 km from release site). The differences in the life-history stategies of these two populations evidently have a genetic basis and are not the product of ecophenotypic variation. The significantly higher sequence and haplotype diversities of Florida Atlantic common snook may be the consequence of their greater vagility (larger neighborhood size (Slatkin 1993)); the similarity between Caribbean snook mtDNA diversities and those of Florida Gulf snook suggests that they share similarities in neighborhood sizes or life-history strategies. In this respect, Florida Atlantic common snook are apparently diverging from other common snook.

Interestingly, the allozyme analysis suggests that Florida Bay snook have transitional genetic properties between east Florida and west Florida snook. A preponderance of evidence suggests that snook do not reproduce within (nor are larvae transported into) the southermost waters of Florida (Peters 1993 and references therein). Thus, snook in the Florida Bay – Florida Keys region likely comprise an immigrant adult population derived from one or both Florida populations. The tagging studies cited above indicate that many snook in the Florida Keys are expatriates of the Florida Atlantic population; however, for the Florida Bay area, the situation remains unclear. A detailed genetic study of snook in south Florida is underway.

The RFLPs detected by *Mbo*I and *Rsa*I provide useful genetic markers for discriminating Florida snook populations from those of the Caribbean. The absence of the B restriction patterns produced by *Mbo*I and *Rsa*I, respectively, in the Caribbean populations sampled suggests that the mutations responsible for those patterns either occurred de novo in Florida or that they have been eliminated from Caribbean populations through lineage sorting or selection. Regardless of the mechanism, the highly significant differences in the frequencies of these patterns are evidence that little genetic exchange presently occurs between snook from the two regions (e.g., Wirgin et al. 1989), despite the overall genetic similarity of west Florida and Caribbean nuclear genomes (mtDNA structure accumulates four times faster than nuclear DNA structure (Birky et al. 1983)).

Gene flow also seems to be restricted in Caribbean island populations. Overall, a comparatively high level of genetic substructuring of heterozygosity (θ) was observed for snook from the Caribbean islands (Puerto Rico and Trinidad) versus snook from other Caribbean locations. The lack of genetic variation in the Trinidad sample reasonably suggests that the population was recently founded by few individuals or has experienced a recent, severe bottleneck. In either case, the Trinidad population must be isolated from others because, in the absence of selection, even low levels of immigration from nearby Venezuela should quickly restore genetic variability (Slatkin 1985).

General implications for fishery management

The results of our study have clear implications for the management of common snook. Throughout the species' range, common snook should be managed at the local or regional level. For example, common snook in Florida are currently managed as if all individuals were members of a single, interbreeding stock. However, the Florida Atlantic and Florida Gulf populations should be managed as separate stocks. It is not likely that a declining population inhabiting one coast would be replenished by recruitment of snook from the other coast. In addition, these two populations differ in the biological characteristics of growth, mortality, and dispersal, confounding the predictive yield models used by fishery managers (Ricker 1973).

The genetic differences we observed in common snook principally reflect shifts in the frequencies of mtDNA haplotypes and allozyme alleles and differing levels of genetic diversity and polymorphism rather than significant geographic groupings of haplotypes and fixed allelic differences. This type of genetic structure seems to be typical for wide-ranging marine and estuarine fishes (e.g., Salini and Shaklee 1988; Gold et al. 1993; Tringali and Wilson 1993) and is probably related to their large but fluctuating population sizes, periodic range expansions and contractions, and migratory and dispersal behaviors. Unlike the mtDNA haplotype distributions of freshwater fishes (e.g., Bermingham and Avise 1986), those of continuously distributed marine fishes are seldom strongly geographically structured, and allozyme alleles rarely show fixed differences between fish from different locations. The process of random drift operates more rapidly in altering the frequencies of pre-existing genotypes than does the process of mutation in producing new genotypes (McMillan et al. 1992), and fluctuations in population abundance can quickly influence the level of genetic diversity within a population (Prodohl et al. 1992). Nevertheless, genotype frequencies and genetic diversities in moderate- to large-sized marine fish populations are generally considered to be temporally stable on a time scale of a decade or more (Wirgin and Waldman 1994). Therefore, genetic stock identification studies that are designed for and based on analyses of genotype frequencies and levels of diversity may be more relevant to managers of marine fisheries than

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those that are based on a phylogenetic approach. This appears to be the case for common snook.

Acknowledgements

We are grateful to R. Taylor and R. Crabtree (Florida Marine Research Institute), C. Alliume (Puerto Rico Department of Natural Resources), B. Fabres and S. Kuruvilla (Ministry of Food Production, Marine Exploitation, Forestry, and the Environment, Trinidad), J. Dean (University of South Carolina), and all others who assisted us in collection of sample specimens. We thank L. French, J. Leiby, T. Perkins, J. Quinn, S. Seyoum, and R. Wilson and two anonymous reviewers for valuable comments that improved the manuscript. We also thank H. Cruz-Lopez and R. Stokes for technical assistance and J. Miller and M. Nei for providing the computer programs RESTSITE and NJTREE, respectively. This study was funded by the federal Sportfish Restoration Act, Department of the Interior, U.S. Fish and Wildlife Service, grant No. F-0296, project F-59, and by the state of Florida.

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Appendix

Enzymes and nonenzymatic proteins and staining procedures used in starch gel electrophoresis of common snook, *Centropomus undecimalis*.

Protein[a]	Enzyme no.	No. of loci	Buffer system	Tissue used	Enzyme structure
Aspartate aminotransferase (*AAT*)	2.6.1.1	3	5	s,l	d
Acid phosphatase (*ACP*)	3.1.3.2	1	5	l	d
Aldehyde oxidase (*AO*)	1.2.3.1	1	2	s,l	?
Creatine kinase (*CK*)	2.7.3.2	3	9	l	m
Diaphorase (*DIA*)	1.8.1.4	2	2	l	d
Esterase (*EST*)	3.1.1.–	2	2	l	m
General protein (*GP*)	—	2	2	s	m
α-Glycerophosphate dehydrogenase (α*GPD*)	1.1.1.8	1	9	s,l	d
Glucose phosphate isomerase (*GPI*)	5.3.1.9	2	3	s	d
Hexanol dehydrogenase (*HDH*)	1.1.1.71	1	3	s,l	?
Isocitrate dehydrogenase (*IDH*)	1.1.1.42	1	9	s,l	d
L-Lactate dehyrogenase (*LDH*)	1.1.1.27	2	3	s,l	t
Malate dehydrogenase (*MDH*)	1.1.1.37	1	5	l	?
Malic enzyme (*ME*)	1.1.1.40	1	6	l	?
Mannose-6-phosphate isomerase (*MPI*)	5.3.1.8	1	2	l	m
Dipeptidase (*PEPA*) (specific for phenylalanyl-leucine)	3.4.–.–	1	8	l	d
Tripeptidase (*PEPB*) (specific for leucyl-glycyl-glycine)	3.4.–.–	1	9	l	d
6-Phosphogluconate dehydrogenase (*6PGD*)	1.1.1.44	1	5	l	?
Phosphoglucomutase (*PGM*)	5.4.2.2	1	8	l	m
Superoxide dismutase (*SOD*)[b]	1.15.1.1	1	3	s,l	?
Xanthine dehydrogenase (*XDH*)	1.1.1.204	1	6	l	d

Note: For locus nomenclature, we followed the recommendations of Shaklee et al. (1990), except we omitted the asterisk from the locus abbreviation. s, somatic muscle; l, liver; m, monomer (presumed heterozygotes show two bands); d, dimer (presumed heterozygotes show three bands); t, tetramer (presumed heterozygotes show five bands).

[a]Most proteins were resolved by the electrophoretic procedures of Selander et al. (1971); DIA was resolved using recipes from Harris and Hopkinson (1976).
[b]Resolved on HDH and ME stains.

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Liming of wetlands in the acidified Lake Røynelandsvatn catchment in southern Norway: effects on stream water chemistry

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Abstract: In 1990, wetlands and blanket bogs, constituting 4.0% of the catchment of Lake Røynelandsvatn in southern Norway, were limed by helicopter with 20 $t \cdot ha^{-1}$ of fine-powdered limestone. Liming caused a rapid increase in stream pH and calcium (Ca) and a decrease in reactive and inorganic monomeric aluminium (Al). Three years later the reactive Al concentration was still 50% lower and the Ca concentration twice as high as preliming values. Mean and minimum acid neutralizing capacity of the outlet stream were 23 and 8 $\mu equiv. \cdot L^{-1}$, respectively, and although pH had decreased to 5.2–5.3 the water quality was acceptable for the survival of brown trout (*Salmo trutta*). No significant increase in stream nitrate, organic nitrogen, or total organic carbon was measured after liming. Terrestrial liming may be a useful approach to neutralize acidic runoff because of the retention of inorganic monomeric Al in the catchment, the long-lasting increase in Ca concentration, and the inherent deacidification capacity during stormflows and snowmelt. Liming of strategic discharge areas in the catchment may limit the extent of undesirable effects, such as the death of *Sphagnum* mosses, on the terrestrial ecosystem.

Résumé : En 1990, des milieux humides et des bogs en couverture formant 4,0% du bassin versant du lac Røynelandsvatn, dans le sud de la Norvège, ont fait l'objet d'un chaulage par hélicoptère. La chaux a été appliquée sous forme d'une poudre fine à raison de 20 $t \cdot ha^{-1}$. Le traitement a causé une augmentation rapide du pH et de la concentration de calcium (Ca) dans le cours d'eau et une diminution des concentrations d'aluminium (Al) monomérique réactif et inorganique. Trois ans après le traitement, les concentrations d'Al réactif était encore 50% plus faible et celles de Ca deux fois plus élevées que les valeurs enregistrées avant le chaulage. Les potentiels de neutralisation de l'acide moyen et minimal de l'émissaire s'établissaient à 23 et 8 $\mu équiv. \cdot L^{-1}$, respectivement, et bien que le pH ait chuté pour se stabiliser autour de 5,2–5,3, l'eau était encore d'une qualité suffisante pour permettre la survie de la truite brune (*Salmo trutta*). Aucune augmentation significative des concentrations de nitrate, d'azote organique ou de carbone organique total n'a été observée dans le cours d'eau après le chaulage. Le chaulage terrestre semble être une approche utile pour neutraliser le ruissellement acide, étant donné la rétention de l'Al monomérique inorganique dans le bassin récepteur et la hausse prolongée des concentrations de Ca et du potentiel de neutralisation de l'acide durant les débits d'orage et la fonte des neiges. Le chaulage des surfaces d'écoulement dans le bassin peut aider à limiter l'ampleur des effets indésirables dans les écosystèmes terrestres, tels la mort des mousses du genre *Sphagnum*.
[Traduit par la Rédaction]

Introduction

Vulnerable areas in Scandinavia, the United Kingdom, and North America have been severely affected by acidification (Likens et al. 1979; Overrein et al. 1980; Wright 1983) because the critical load for strong acids has been exceeded. Although many European countries have agreed to reduce sulphur (S) levels by 70–80% by the year 2010 relative to 1980 levels (United Nations 1994), acidification will still be a problem for many decades in large areas of Norway (Henriksen and Hindar 1993). Countermeasures such as liming are one way to improve the conditions for aquatic life to restore and maintain the number of sensitive aquatic species in these areas.

Lake liming, stream liming, catchment liming, or a combination of these strategies have been used with positive results in Scandinavia and North America during the last 10–20 years (Rosseland and Hindar 1988; Hindar and Rosseland 1991; Olem 1991; Porcella et al. 1991; Thörnelöf and Degerman 1991; Henrikson and Brodin 1995). Some undesirable effects such as extreme growth of *Juncus bulbosus* in shallow shore areas (Roelofs et al. 1994) and dieback of *Sphagnum* species in wetlands (Mackenzie 1992) have been documented.

Liming of lakes and streams neutralizes acidic water, adds calcium (Ca), and reduces toxic inorganic aluminum (Al) to negligible levels (Wright 1985; Rosseland and Hindar 1988; Grieve 1990; Hindar and Henriksen 1992; Henrikson and Brodin 1995). However, Al-rich, acidic meltwater entering ice-covered limed lakes reacidifies nearshore areas. This is an annual problem in many limed lakes (Hindar and Rosseland 1991; Porcella et al. 1991; Gubala et al. 1992) and thereby a disadvantage of lake liming. Also, highly toxic mixing zones may arise (Rosseland and Hindar 1991; Rosseland et al. 1992). These zones are characterized by dynamic Al chemistry and

Received March 15, 1995. Accepted November 20, 1995.
J12817

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may be even more toxic to fish than the acidic water itself. Therefore, snowmelt may still be a problem for aquatic organisms in traditionally limed lakes, and alternative or supplementary mitigation methods may be needed.

Many practical problems may limit liming of acidified watercourses. Some lakes are difficult to reach by ordinary lime-dosing equipment, electricity may not be available for lime dosing, and inlet streams may not be suitable for dosing. Because of the short retention times for many Norwegian lakes (<0.1–0.3 years), liming twice a year or more often may be necessary.

Negative effects of terrestrial liming may include increased mineralization of organic matter and nitrogen (N) mobilization (Matzner et al. 1985; Persson et al. 1989). Westling and Skärby (1993) showed increased N leakage after liming of forest soil in areas with high N deposition. Thus, increased stream concentrations of inorganic and organic N species may be expected. After whole-catchment liming at Tjønnstrond, Norway, the stream NO_3 concentration increased markedly (high levels relative to the natural variation in a nearby reference area) but returned to its former levels 1–2 years later (Traaen 1991).

The experimental liming of wetlands in the acidified Lake Røynelandsvatn catchment focuses on both aquatic and terrestrial effects. In this paper, changes in water chemistry and fish toxicity are discussed. This liming strategy is compared with other terrestrial liming approaches and with liming directly on the lake surface.

Materials and methods

The catchment

The 1.3-km^2 catchment of Lake Røynelandsvatn is situated 220–300 m above sea level in Aust-Agder County, southern Norway (Fig. 1). The bedrock is a slowly weathering granite overlain by thin and patchy soils with a more or less continuous stretch of *Sphagnum* bogs and marshland underlain by moraine in the depressions. The hills are partly covered with Scots pine (*Pinus sylvestris*) and heather (*Calluna vulgaris*), the northern slopes with Norway spruce (*Picea abies*), and the southern slopes mainly with oak (*Quercus* spp.) and birch (*Betula pubescens*).

Atmospheric wet deposition rates of SO_4 and $NH_4 + NO_3$ in 1990 at two nearby monitoring stations were 40 mmol S·m^{-2}·year^{-1} and 110–120 mmol N·m^{-2}·year^{-1}, respectively (Norwegian State Pollution Control Authority 1991).

The lake

Lake Røynelandsvatn has a lake area of 3.5 ha, is shallow with a maximum depth of 7.5 m and mean depth of 3.5 m, and has a retention time of only 0.094 years (35 days). The lake is normally ice covered from November until May. Because of the short retention time, in-lake processes are supposed to be of minor importance to outlet stream water chemistry on a yearly basis, although the lake water itself may undergo changes (as clearing resulting from net sedimentation of Al–humic complexes and increased pH owing to CO_2 consumption by algae) during the dry parts of the summer.

In southern and northern areas the lake is surrounded by *Sphagnum* bogs. Stands of *Juncus bulbosus* have been observed in shallow parts of the lake, but this weed has not increased to form dense mats as has been observed after liming of some lakes in southwestern parts of Norway (Roelofs et al. 1994).

Hydrology

Runoff was measured every 15 min at a discharge gauging station at the lake inlet (Fig. 1). Data were automatically collected and stored at the site. Specific runoff is 30 L·s^{-1}·km^{-2} in the area as a mean for the 30-year period 1930–1960 (Norwegian Water Resources and Energy Administration 1987). Because of thin soils and barren rocks in most of the catchment, runoff peaks sharply during rain events, and the catchment has a low water-retention capacity.

Liming

In August 1990, a 5.1-ha area, constituting 4.0% of the lake catchment, was limed by helicopter with 103 t of fine-powdered calcitic limestone: 90% <0.064 mm, 50% <0.010 mm, and 20% <0.002 mm, with 31% Ca, 6.5% Si, and 0.8% Mg. The limed areas (areas A–F, Fig. 1) covered an almost continuous *Sphagnum*-dominated bog along the inlet stream. Discharge areas (outflow dominated) and ombrotrophic and flooded bogs were chosen (Table 1). The lime dose for all treated areas was 20 t·ha^{-1}, which corresponds to 0.79 t·ha^{-1} for the entire catchment.

Water sampling and analyses

Two sampling points within reference areas (REF 1 and REF 2) and two sampling points within the limed area (LAK 1 and LAK 2) were included in the monitoring programme (Fig. 1). LAK 1 is located at the inlet and LAK 2 at the outlet of Lake Røynelandsvatn. Samples were collected monthly and more frequently (weekly or biweekly; only pH, alkalinity, and Ca concentration) in periods with moderate to high flow to get representative Ca concentrations for transport calculations. Sampling at peak flow was difficult because of the rapid variation in flow during rain episodes, but data from intensive sampling during flow episodes indicated relatively minor variations in Ca concentrations at moderate to high flow.

Chemical analyses were performed by Norwegian standard methods at the laboratory of NIVA (Norwegian Institute for Water Research), Oslo. Both alkalinity and calculated acid-neutralizing capacity (ANC) are included in this paper: alkalinity as a measure of stream buffering capacity after liming and ANC as a measure of water quality in relation to fish status (Lien et al. 1992; Bulger et al. 1993). Alkalinity was analyzed by potentiometric titration to pH 4.5 (ALK 4.5) and corrected according to Henriksen (1982) to give equivalence-point alkalinity (ALK-E):

$$\text{ALK-E } (\mu\text{equiv.} \cdot \text{L}^{-1}) = \text{ALK } 4.5 - 32 + 0.646(\text{ALK } 4.5 - 32)^{0.5}$$

Negative values may be obtained when ALK 4.5 is less than 32 μequiv.·L^{-1}. ANC was calculated as

$$\text{ANC} = Ca^{2+} + Mg^{2+} + Na^{+} + K^{+} - (SO_4^{2-} + Cl^{-} + NO_3^{-})$$

where all concentrations are in microequivalents per litre. According to the charge balance and on the basis of the assumption that the concentrations of CO_3^{2-}, OH^-, and NH_4^+ are negligible, ANC can also be calculated as $\text{ANC} = A^- + HCO_3^- - (H^+ + \Sigma Al^{n+})$, where A^- is the concentration (in microequivalents per litre) of organic anions and ΣAl^{n+} is the equivalent sum of all positively charged Al species (see Reuss and Johnson 1986).

Reactive Al (RAl) includes both the inorganic monomeric Al fraction (Al_i) and the organic monomeric Al fraction (Al_o). These fractions were separated by ion exchange.

Organic N is defined as total N - NO_3^- - NH_4^+, where all concentrations are in micrograms N per litre. Two-way analysis of variance on log-normalized values was used to test the significance of changes in the concentrations of NO_3, organic N, and total organic carbon (TOC) following liming.

Calculations of lime transport were based on the net increase in Ca concentration relative to the calculated mean background concentration of Ca at LAK 1 (Table 2), runoff, and the composition of the limestone material.

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Fig. 1. The catchment of Lake Røynelandsvatn. Bogs are hatched by horizontal lines and limed areas are marked with capital letters (see Table 1) and by oblique hatching. Data from the two reference stations (REF 1 and REF 2) together with data from the main inlet (LAK 1) and the outlet (LAK 2) of the lake are presented. Runoff was measured at a discharge gauging station at the lake inlet (LAK 1).

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Table 1. Characteristics of the limed bogs and lime doses.

Bog characteristics	Limed area (ha)	Catchment (ha)	Lime dose (t)
(A) Mainly ombrotrophic	1.25	12.9	25.0
(B) Outflow	0.51	11.2	10.2
(C) Outflow	0.25	35.3	5.0
(D) Outflow	0.51	4.5	10.2
(E) Outflow	0.45	10.3	9.0
(F) Ombrotrophic, flooded	2.17	84.6	43.4
Total	5.14		102.8

Note: Capital letters refer to limed areas shown in Fig. 1.

Table 2. Basic water chemistry (mean ± SD) at the lake inlet (LAK 1) and lake outlet (LAK 2) before (August 1988 – August 1990) and after (yearly mean) liming.

	Inlet (LAK 1)				Outlet (LAK 2)			
	Before (n = 12,12)	1991 (n = 40,28)	1992 (n = 63,14)	1993 (n = 9,9)	Before (n = 11,11)	1991 (n = 25,19)	1992 (n = 18,13)	1993 (n = 9,9)
pH	4.51	6.43	6.13	5.51	4.57	6.16	5.88	5.24
H^+ (μequiv.·L^{-1})	31±12	0.4±0.2	0.7±0.5	3.1±0.7	27±10	0.7±0.3	1.3±0.9	5.7±1.4
ALK-E (μequiv.·L^{-1})		125±37	99±106	35±17		67±24	31±9	5±2
Ca (μequiv.·L^{-1})	51±15	242±41	206±39	165±26	43±11	185±24	136±13	128±7
Mg (μequiv.·L^{-1})	31±11	33±4	32±7	40±7	31±7	33±4	29±2	39±4
K (μequiv.·L^{-1})	6±5	7±5	7±4	6±4	7±2	6±1	6±1	7±2
Na (μequiv.·L^{-1})	122±27	139±16	133±17	180±13	120±22	149±21	132±12	177±8
SO_4 (μequiv.·L^{-1})	109±32	105±16	105±19	103±20	102±17	95±13	98±9	103±17
Cl (μequiv.·L^{-1})	138±40	142±33	132±27	210±53	138±32	166±39	134±16	210±28
NO_3 (μequiv.·L^{-1})	9±10	32±14	12±8	17±10	12±8	24±14	13±7	17±9
Total N (μg·L^{-1})	428±211	795±340	469±116	485±175	389±155	632±262	451±89	483±127
RAl (μg·L^{-1})	398±122	142±30	204±55	240±60	322±71	143±35	121±48	197±46
Al_i (μg·L^{-1})	302±121	32±10	30±21	64±42	286±70	34±15	17±8	76±19
TOC (mg·L^{-1})	5.8±1.6	6.0±2.4	9.7±4.0	6.2±3.2	3.8±1.8	5.3±1.5	6.8±0.6	5.0±1.8
ANC (μequiv.·L^{-1})	−44±26	154±44	119±44	62±21	−50±26	94±25	55±20	23±9

Note: The values for n are given as two numbers: the first is the number of pH, alkalinity, and Ca measurements; the second is the number of measurements of the other parameters.

Results

Basic water chemistry data from before (August 1988 – August 1990) and 3 years after liming are given in Table 2. Prior to liming, the Røynelandsvatn catchment was chronically acidified. The annual average pH at the outlet of the lake was 4.55 (H^+ concentration: 28 ± 10 μequiv.·L^{-1}), while the concentrations of Ca, SO_4, and Al_i were 43 ± 11 μequiv.·L^{-1}, 102 ± 17 μequiv.·L^{-1}, and 286 ± 70 μg·L^{-1}, respectively. These are typical concentrations for acidified areas in southern Norway. The TOC at the lake outlet was 3.8 ± 1.8 mg C·L^{-1}, markedly lower than that of both the lake inlet (5.8 ± 1.6 mg C·L^{-1}) and the two reference streams (6.3 ± 2.5 and 8.2 ± 3.3 mg C·L^{-1}, respectively). This difference may be explained by in-lake retention of dissolved organic matter. The ANC was −50 ± 26 μequiv.·L^{-1}, indicating major exceedance of the critical load for brown trout (*Salmo trutta*) (see Lien et al. 1992).

As a result of liming, pH increased to target values of between 6 and 7 and the Ca concentration increased to about 250 μequiv.·L^{-1} 1 year after treatment (Fig. 2). ANC increased to 200–300 μequiv.·L^{-1} in autumn 1990 and was 50–200 μequiv.·L^{-1} in 1991. RAl was quickly reduced to about one third of preliming values, and the concentrations of Al_i approached zero (Fig. 3).

Lake Røynelandsvatn reacidified gradually and outlet stream pH was 5.2–5.3 at the end of 1993, 3 years after liming (Fig. 2). However, Al_i was still relatively low at 50–70 μg·L^{-1} Al and RAl was 50% below the RAl of the untreated reference areas and preliming values (Fig. 3). Also, alkalinity of both inlet and outlet streams was positive throughout the period, except for a sea-salt episode (February 1993, see below) when alkalinity decreased to −1 μequiv.·L^{-1} at the lake outlet. As discussed later, stream-buffering capacity after liming was higher than indicated by pH. The mean Ca concentration in 1993 was 128 μequiv.·L^{-1}, more than twice the preliming value. In 1993, annual mean and minimum ANC at the lake outlet were 23 and 8 μequiv.·L^{-1}, respectively. According to Lien et al. (1992), these results indicate acceptable water quality for brown trout.

Slightly lower pH, Ca concentrations, and ANC and a slightly higher Al concentration were present in the outlet of Lake Røynelandsvatn compared with the inlet stream. This was directly related to the influence of acid water derived from unlimed parts of the catchment (representing 30% of the total catchment) and acid deposition directly on the lake surface. However, there were no signs of highly acidic, Al-rich meltwater flushing through the lake during periods with ice cover (Fig. 3).

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Fig. 2. Variations in pH, Ca concentration, and ANC at the two reference stations (REF 1 and REF 2) and at the inlet (LAK 1) and outlet (LAK 2) of Lake Røynelandsvatn.

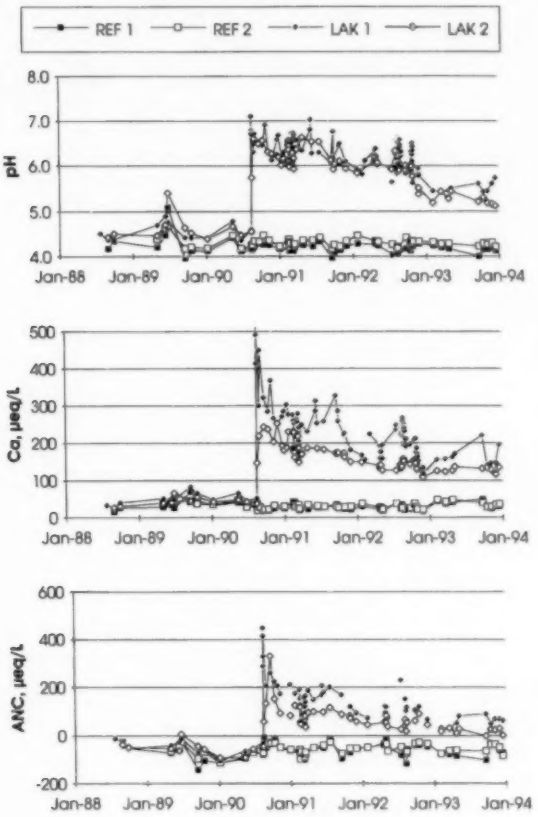
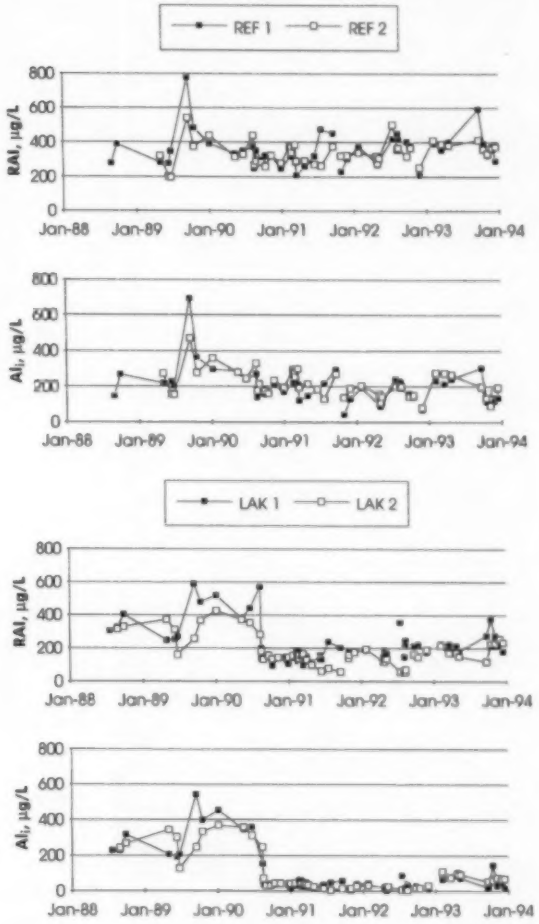


Fig. 3. Variations in reactive (RAl) and inorganic monomeric (Al_i) aluminium at the two reference stations (REF 1 and REF 2) and at the inlet (LAK 1) and outlet (LAK 2) of Lake Røynelandsvatn.



In periods with low flow, the pH in the inlet stream was low relative to the stream-buffering capacity (alkalinity) and Ca concentration. Most likely, this can be attributed to increased concentrations of CO_2 when runoff was dominated by groundwater and water passing through the organic-rich wetland soils. Dissolved CO_2, although not measured routinely, played an important role in the variation in stream pH. At low flow, experimental degassing of wetland runoff samples resulted in pH increases of 0.5–1.0 pH units. The pH typically increases by 1 pH unit after CO_2 degassing when $pCO_2 > 10^{-2.5}$ atm (1 atm = 101.3 kPa) (Norton and Henriksen 1983; Small and Sutton 1986), i.e., at more than 10 times the atmospheric CO_2 pressure. Accordingly, pH is a relatively poor indicator of the buffering capacity especially at low flow when the oversaturation of CO_2 is probably highest.

Slightly higher stream NO_3 concentrations were found in the limed catchment than in the two reference catchments the first summer. This difference did not continue the following year. The NO_3 concentrations of the treated areas had been within natural variations for the area (Fig. 4). TOC increased in the period after liming, but a parallel increase of the same magnitude was also found in the two reference areas. There were significant differences ($P < 0.001$) in NO_3 and TOC between the stations. Among years (1989–1993) there were significant differences in NO_3 concentrations ($P < 0.001$), organic N concentrations ($P < 0.05$), and TOC ($P < 0.005$). However, none of these changes can be explained as an effect of liming because there were no significant two-way interactions in the ANOVA test.

A sea-salt episode in January 1993 (Hindar et al. 1994) resulted in a relatively long-lasting (about 8 months) increase in Na and Cl levels and thereby increased the yearly mean concentrations of these ions at both the inlet and outlet monitoring stations (Table 2). The elevation in the concentrations of Na and Cl until September may also have been due to the very dry conditions in this period (Fig. 5) and poor conditions for reestablishing the prestorm composition of soil water. This episode was the most severe sea-salt episode in southern Norway in decades and resulted in nonmarine Na concentrations (relative to Cl) of –38 and –20 µequiv.·L^{-1} at LAK 1 and LAK 2, respectively, in February 1993. However, no dramatic

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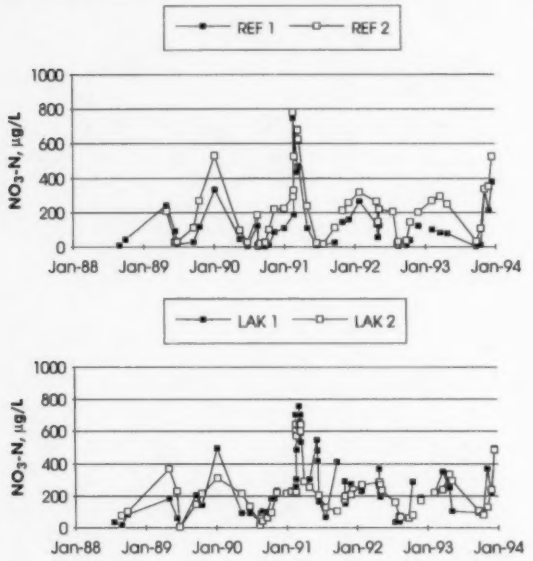
Fig. 4. Variations in nitrate (NO_3-N) at the two reference stations (REF 1 and REF 2) and at the inlet (LAK 1) and outlet (LAK 2) of Lake Røynelandsvatn.



changes in pH, ANC, or toxic Al_i levels at Røynelandsvatn (Figs. 2 and 3) were observed.

Weekly mean values of runoff at LAK 1 are shown in Fig. 5. As daily and even weekly runoff values vary by several orders of magnitude and the concentration of Ca is relatively stable after the initial increase, the hydrology of the catchment is an indicator of how fast dissolved lime will be transported from the limed areas and out of the lake. The driest year of the research period was 1993. Practically no runoff (weekly mean value <3 L·s^{-1}) was measured during a 15-week period from May to September that year. Mean specific runoff was more than twice as high (74 L·s^{-1}·km^{-2}) as normal in the year of liming (1990) and 37, 30, and 21 L·s^{-1}·km^{-2} for the next 3 years. It is, therefore, not surprising that a relatively large fraction of the applied limestone, about 35%, was transported out of the catchment during the first 1.5 years (Fig. 6) and only 11% during the next 2 years. However, declining Ca transport during the period also indicates that less limestone was available for deacidification at the end of the period, as is also seen from the decreasing pH. The wetland liming severely affected *Sphagnum* mosses on the limed areas, which apparently lost chlorophyll and died.

Discussion

Liming may be an appropriate countermeasure to maintain or restore the number of sensitive aquatic species of acidified waters until S emissions are sufficiently reduced (Henrikson and Brodin 1995). However, even after dramatic decreases in acid deposition, liming may still be necessary in the most acid-sensitive areas in Norway (Henriksen and Hindar 1993).

Liming strategies should be based on the biological targets and ecological effects desired. The need for several liming options and careful planning is obvious. The potential advantage of terrestrial liming is a more stable surface water chemistry, whereby acidic, Al-rich meltwater may be neutralized before it enters a target lake or stream. Therefore, episodic reacidification of the nearshore areas should be avoided. This and other studies show that whole-catchment liming, liming of hydrologic source areas, and blanket bog liming achieve these goals (Rosseland and Hindar 1988; Brown et al. 1988; Grieve 1990; Gubala and Driscoll 1991; Jenkins et al. 1991; Stoner and Donald 1991). Liming of wetlands may dramatically limit the total liming area compared with whole-catchment liming, thereby reducing the extent of unwanted effects, and hence it may be more acceptable.

The success of different liming techniques such as lake liming, lime dosing, and terrestrial liming depends both on the desirable versus undesirable ecological effects and the cost. Whereas lime application directly on surface water only affects the aquatic ecosystem, terrestrial liming also introduces changes in other ecosystems. Desirable effects include improved water quality and improved living conditions for aquatic organisms, whereas undesirable effects may include the dieback of *Sphagnum* species and changes in wetland structure and function. Limestone dissolution is fairly high after both lake and stream liming but may be lower after terrestrial liming (Hindar and Rosseland 1991; Henrikson and Brodin 1995), thus increasing costs. However, if the overall ecosystem features are significantly improved by catchment liming, higher costs may be acceptable.

Terrestrial liming may be further divided into three types: (*i*) forest liming, (*ii*) whole-catchment liming for aquatic effects, and (*iii*) wetland liming. It is important to distinguish among these techniques. Forest liming is usually characterized by relatively low doses of coarse (0.2–2 mm) Mg-containing limestone or dolomite (Larsson and Westling 1993; Kreutzer 1994). The primary objectives are increased pH, base saturation, and Mg availability in forest soils. Whole-catchment liming to improve stream water quality may be restricted to areas of thin soil to ensure a sufficient response as in Tjønnstrond (Rosseland and Hindar 1988) and may be long lasting (10 years). Liming of hydrologic source areas or wetlands requires high doses of calcitic limestone powder spread on a limited area, as in this study and as shown by Jenkins et al. (1991). The effect on water quality may be relatively short (1–5 years).

Achievement of an acceptable water quality for fish and other sensitive aquatic organisms is the major goal of liming. Although Al is the primary toxic element, pH, Ca concentration, and TOC are also important for evaluating water toxicity (Wood and McDonald 1987; Brown and Sadler 1989; Rosseland et al. 1990). Increased Ca concentrations may offset the toxic effects of inorganic monomeric Al (Muniz and Leivestad 1980; Brown 1983). Al is present in many physicochemical forms depending on pH, temperature, and levels of organic and inorganic ligands. The recommended water quality for salmonid fish in waters with low Ca concentrations (<2 mg·L^{-1}) specifies that the concentration of Al_i should not exceed 15–30 µg·L^{-1}, whereas for waters with Ca concentrations >5 mg·L^{-1} the Al_i concentrations should not exceed 60–75 µg·L^{-1} (Howells et al. 1990). In waters with high levels of dissolved organic carbon (>10 mg·L^{-1}), higher Al_i concentrations could be accepted. Seasonal temperature variations in

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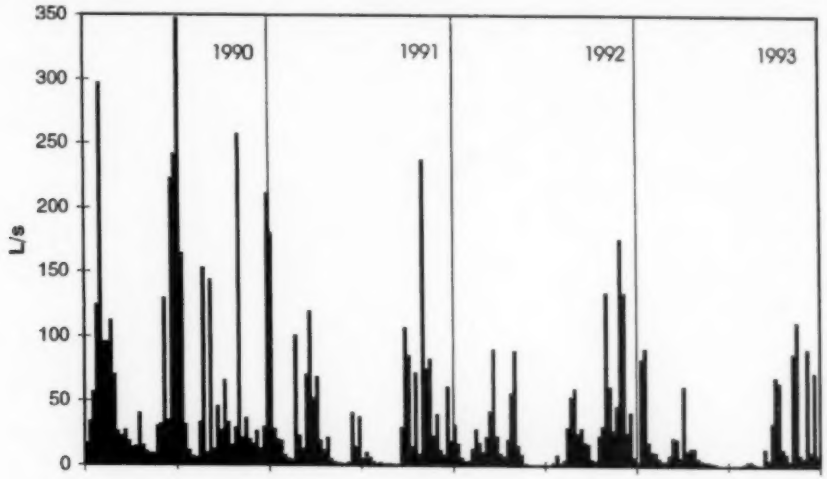
Fig. 5. Weekly mean values of runoff at the discharge gauging station at the lake inlet (LAK 1) for the period 1990–1993.



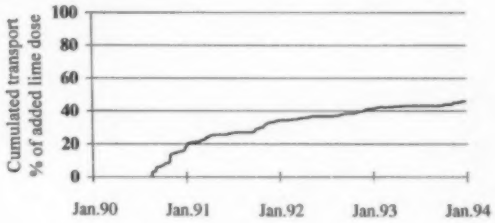
surface waters (0–20°C) may also change the Al toxicity along the pH scale (Lydersen 1990), thus increasing the complexity.

The increased pH, alkalinity, and Ca concentrations, the relatively long-lasting decrease in the concentration of reactive Al, and the low concentrations of toxic inorganic monomeric Al show that the liming strategy at Røynelandsvatn was successful for some time. An evaluation of the water quality, as related to fish status and based primarily on ANC (Lien et al. 1992; Bulger et al. 1993) and Al_i, indicates that the conditions of the stream and lake water were acceptable for the survival and reproduction of brown trout in 1993, 3 years after liming. No test fishing was included in the research programme, but a brown trout population was established in the lake, probably as a result of the wetland liming, successful stocking, and probably also migration from a limed lake downstream. The most acid-sensitive aquatic organisms do not survive at pH values below 6.0. If present at the time of liming, they would not have been protected for more than 2 years after treatment.

A long-lasting influence on RAl, from preliming values of 100–300 $\mu g \cdot L^{-1}$ down to 20–100 $\mu g \cdot L^{-1}$ following liming, compared with pH has also been reported after whole-catchment liming at Tjønnstrond (Traaen 1991). Thus, liming of wetlands (Røynelandsvatn) and other terrestrial parts of a catchment (Tjønnstrond) seems to affect Al retention capacity for a longer period than indicated by stream water pH.

An important result of the wetland liming strategy was the stability of the water quality at the lake outlet even during snowmelt and fall spates. This stability was also observed after liming of Woods Lake, New York (Gubala and Driscoll 1991), and Loch Fleet, Scotland (Dalziel et al. 1992). No signs of acidic, Al-rich water were found when meltwater flushed through Lake Røynelandsvatn in periods with ice cover. This was also true during the sea-salt episode in winter 1993, which resulted in a dramatic increase in water toxicity in unlimed watercourses (Hindar et al. 1994). Thus, sensitive organisms in the ecologically important littoral zone of the lakes may be protected. At increasing bog water levels the importance of

Fig. 6. Cumulated transport of lime as calculated from net Ca concentrations and runoff. Data are given as percentages of the total lime dose.



surface and subsurface flow probably increased. This in turn improved the contact between throughflow and limed bog surfaces. The resulting maintenance of stable pH and Ca concentration at relatively high levels ensured acceptable water quality for aquatic organisms even at moderate to high flows. Therefore, limed wetlands may serve as a dynamic lime-dosing system, adding lime when needed. At Røynelandsvatn, only 4.0% of the catchment was limed with approximately the same dose as for Loch Fleet (20 $t \cdot ha^{-1}$), and comparable water-quality stability was achieved.

Removal of Al probably occurred mainly on limed *Sphagnum* and soil surfaces and thus was restricted to limited areas (4.0%) of the catchment. Runoff from unlimed parts of the catchment was in limited or no contact with limed areas before mixing with high-pH water in the stream. Inflowing water to the limed area will gradually erode and dissolve calcite particles, thus gradually decreasing the deacidifying capacity. The pH increase will also gradually change the dissolved Al species in the limed areas from predominantly Al^{3+} (at pH <4.5–5.0) to formation of lower cationic $(Al(OH)_n^{(3-n)+})$ polymers that may precipitate more easily. With aging, the reactivity of Al retained in topsoil and moss surfaces will be further reduced, which means that the remobilization of Al is also reduced. Finally, the rapid flush rate during high flow

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does not secure complete removal of Al from the water flushing over the limed surfaces because of the relatively slow polymerization and precipitation rates. Low temperatures from late autumn (November) until May slow these reaction rates even more. This may explain why some Al was always present in the stream water. Investigating the Al-removing capacity of surface soils and *Sphagnum* mosses may reveal the Al dynamics after wetland liming.

No significant increase in NO_3^- was found after liming at Lake Røynelandsvatn. Mineralization of wetland soils and mosses may be too slow to produce a significant increase in stream N concentration. This is in contrast with the short-term increase in NO_3^- after whole-catchment liming at Tjønnstrond (Traaen 1991). No significant change in organic N concentration or TOC indicates that increased mineralization of the organic soils did not occur, or it was too slow to appear as changes in stream water composition, or C and N were released as gases.

Lower lime doses and more frequent application, increased grain size, and probably also introduction of Mg-containing dolomitic limestone may reduce such undesirable terrestrial effects as the dieback of *Sphagnum* mosses. However, more cautious use of neutralizing agents may reduce the beneficial aquatic effects. Further, long-term effects on bog structure and function are not yet documented as repeated liming for decades may be necessary to achieve such changes. We believe further research is required before final recommendations on wetland liming may be provided.

Conclusions

Wetland liming aiming to improve stream and lake water quality at Røynelandsvatn was characterized by the following features. (*i*) On the basis primarily of pH, ANC, and Al_i concentrations, the water quality after liming was stabilized at acceptable levels for salmonids for about 3 years and for more sensitive aquatic organisms (if present at the time of liming) for about 2 years. (*ii*) Limed wetlands may serve as a dynamic lime-dosing system, adding lime when needed. (*iii*) No episodic acidification occurred in the lake after liming, and this reduced toxic conditions for sensitive aquatic organisms in the littoral zone. (*iv*) A large fraction of the mobilized Al from the acidified catchment was either adsorbed or precipitated in the limed area and did not reach the stream or lake. This effect may last longer than indicated by pH. (*v*) Wetland liming did not result in soil and moss mineralization that could affect stream water chemistry. (*vi*) Desirable effects should be weighed against undesirable terrestrial effects, such as the dieback of *Sphagnum* species in the limed areas. (*vii*) Although wetland liming restricts the extent of undesirable effects of terrestrial liming, this deacidification technique requires further development.

Acknowledgements

This work is part of the research and monitoring in conjunction with the Norwegian national liming programme and was partly financed by the Directorate for Nature Management (contract 66203/93) and by the Research Council of Norway. We thank Dr. Richard F. Wright for a critical review of the manuscript.

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Biomass-size spectra and the prediction of fish biomass in lakes[1]

Hélène Cyr and Robert H. Peters

Abstract: Biomass-size spectra of planktonic communities offer a promising approach to predict fish biomass from simple measurements of phytoplankton or zooplankton biomass. Theoretical models based on the transfer of energy between size-classes have predicted fish biomass in the Great Lakes within two fold of measured biomass. To test the applicability of one group of biomass-size spectrum models (U. Borgmann 1987. Can. J. Fish. Aquat. Sci. 44(Suppl. 2): 136–140) to lakes in general, we gathered biomass and production data for phytoplankton, phytobenthos, zooplankton, zoobenthos, and fish from 24 lakes and reservoirs studied during the International Biological Programme. In planktonic communities, the intercepts of biomass-size spectra are positively related to phytoplankton productivity and to lake mean depth, and the slopes become more negative with increasing lake size. Despite these regularities, fish biomass is predicted poorly from Borgmann's biomass-size spectrum models. Predictions of fish biomass were only weakly correlated to measured fish biomass ($r^2 = 0.19$, $n = 24$). Fish biomass was overestimated or underestimated by more than 50 kg·ha^{-2} in almost half the lakes where the models were tested. The inclusion of benthic algae and benthic invertebrates in the models does not improve the predictions of fish biomass.

Résumé : Les spectres de la biomasse en fonction de la taille de communautés planctoniques ouvrent une avenue intéressante pour la prévision de la biomasse du poisson à partir de mesures simples de celle du phytoplancton ou du zooplancton. Des modèles théoriques fondés sur les transferts énergétiques entre les différentes classes de taille ont permis de formuler des prévisions de la biomasse du poisson des Grands Lacs qui correspondaient à deux ordres de grandeur près aux calculs fondés sur des mesures de la biomasse. Afin de tester l'applicabilité aux lacs en général d'un groupe de modèles fondés sur les spectres de la biomasse en fonction de la taille (U. Borgmann 1987. J. Can. Sci. Halieut. Aquat. 44(Suppl. 2): 136–140), nous avons amassé des données sur la biomasse et la production du phytoplancton, du phytobenthos, du zooplancton et du zoobenthos ainsi que sur celles des poissons dans 24 lacs étudiés dans le cadre du Programme biologique international. Chez les communautés planctoniques, il existe une corrélation positive entre les coordonnées à l'origine des spectres de la biomasse en fonction de la taille et la productivité phytoplanctonique ainsi que la profondeur moyenne des lacs, et plus le lac est grand, plus la pente de la courbe est négative. Malgré ces tendances constantes, les modèles de Borgmann prédisent mal la biomasse du poisson. Il n'existait qu'une mince corrélation entre les prévisions et la biomasse mesurée ($r^2 = 0{,}19$, $n = 24$). Celle-ci était sur- ou sous-évaluée de plus de 50 kg·ha^{-2} dans presque la moitié des lacs qui ont servi aux essais avec les modèles. L'inclusion dans les modèles des algues et des invertébrés benthiques n'a pas permis d'améliorer les prévisions relatives à la biomasse du poisson dans les lacs.
[Traduit par la Rédaction]

Introduction

When Sheldon et al. (1972, 1977) first suggested that phytoplankton, zooplankton, and fish reach about equal biomass in marine plankton, they provided a new approach to predict fish biomass in the ocean. Models of energy transfers among trophic levels were soon developed that reproduced Sheldon's observations and offered a theoretical framework, and for many legitimacy, for the equal-biomass hypothesis (Kerr 1974; Sheldon et al. 1977). This work generated considerable interest in aquatic ecology. More data were collected in marine and freshwater environments, providing further evidence of patterns in biomass-size spectra (e.g., Schwinghamer 1981; Sprules and Munawar 1986; Ahrens and Peters 1991; Cattaneo 1993). The models were also extended to predict dynamic variables (e.g., fish production, contaminant cycling; Borgmann 1982; Griesbach et al. 1982; Borgmann and Whittle 1983; Sprules et al. 1991) and to reproduce the finer dome-shaped structure of the observed biomass-size spectra (Thiebaux and Dickie 1992).

Two closely related groups of biomass-size spectrum models have been developed. One group of models is based on energy transfers from prey to predators (e.g., Platt and Denman 1978; Silvert and Platt 1978; Thiebaux and Dickie 1992,

Received March 28, 1995. Accepted November 20, 1995.
J12840

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1993), and the other group is structured around energy transfers between organisms of different sizes, on the basis of the precept that the trophic structure of aquatic communities is closely related to the size of organisms (Borgmann 1982, 1987). These models have enough flexibility to predict the biomass and ecological rates of any size group of organisms, and although predictions from either group of models have seldom been tested, the results are encouraging (Paranjape and Sheldon 1991). Predicted fish biomass and zooplankton production were within two fold of the observed values in the Bay of Quinte, Lake Ontario (fish; Minns et al. 1987), in the open water of Lake Ontario (zooplankton; Sprules and Goyke 1994), and in Lake Michigan (planktivorous fish; Sprules et al. 1991). Biomass-size spectra thus offer a promising approach to aquatic ecology (Evans et al. 1987; Leach et al. 1987; Dickie et al. 1987).

To date, biomass-size spectrum models have been used to predict fish biomass or production in the ocean and in very large lakes. Several factors may complicate their application to smaller lakes. The importance of structures (e.g., macrophytes, stream inlets, fallen trees, shoreline features) and the relatively small volume of most lakes suggest that biomass-size spectra may be more spatially and temporally variable in lakes than in the oceans. Moreover, pelagic communities in small lakes may receive substantial inputs of organic matter (food) from the littoral zone and from the surrounding land (Cole et al. 1994; del Giorgio and Peters 1994). These allochtonous sources of food should be included in models but are almost impossible to quantify. Despite these complexities, regular biomass-size spectra are found in many lakes, at least from bacteria to large zooplankton (e.g., Sprules and Munawar 1986; Rodriguez et al. 1990; Ahrens and Peters 1991; Gasol et al. 1991; Gaedke 1992) and perhaps to fish (Sprules et al. 1983). Moreover, Minns et al. (1987) tested a biomass-size spectrum model in different parts of a large but shallow bay (9.7 m mean depth, 257 km^2 surface area) connected to Lake Ontario and found good agreement between predicted and measured fish biomass. These results suggest that few adjustments may be necessary to apply biomass-size spectrum models to lakes in general.

An added complication in lakes is the interaction between planktonic and benthic organisms. Biomass-size spectrum models applied to the oceans and to large lakes have focussed on planktonic communities, but in most lakes, planktonic communities are not isolated from benthic communities. Planktonic algae sink and provide food to benthic invertebrates, benthic invertebrates feed on zooplankton, and fish feed both on planktonic and on benthic invertebrates. Schwinghamer (1985) proposes that such interactions are reflected in the complementary peaks and troughs observed in planktonic and in benthic biomass-size spectra. Boudreau and Dickie (1992) further suggest that biomass-size spectra that include both planktonic and benthic organisms have constant slopes over a wide range of environments. These studies imply that biomass-size spectra of whole lake communities should be more regular than those based solely on planktonic organisms and would be better predictors of fish biomass. The use of benthic organisms in biomass-size spectrum models remains to be tested.

An appealing feature of biomass-size spectra is their constancy. Different models are based on primary (overall) slopes of biomass-size spectra between 0 (equal biomass) and –0.22. Measured slopes, however, vary from 0.25 to –0.50 in different lakes (Sprules and Munawar 1986; Rodríguez et al. 1990; Ahrens and Peters 1991; Gasol et al. 1991; Gaedke 1992), from 0.23 to –0.24 in the Great Lakes (Sprules and Munawar 1986), and from 0.01 to –0.16 even in the ocean (Witek and Krajewska-Soltys 1989; Sprules and Munawar 1986). Some of this variability may be methodological, since most studies focus on partial spectra that do not include fish (Boudreau and Dickie 1992). More interestingly, part of this variability may be due to differences in site characteristics. The slopes of biomass-size spectra have been reported to vary systematically with total phosphorus concentration in the water, a measure of lake productivity (Sprules and Munawar 1986; Ahrens and Peters 1991). Such general relationships between biomass-size spectra and site characteristics may help refine the models for particular sites. So far, however, comparisons of biomass-size spectra have been limited to a few sites and seldom include fish biomass.

We use the extensive biomass and production data collected in lakes throughout the world during the International Biological Programme (IBP) to compare the primary structure of biomass-size spectra (sensu Dickie et al. 1987) across a wide range of lakes and to test fish biomass predictions made from some of these models. We first test whether the slopes and intercepts of biomass-size spectra vary systematically with simple lake characteristics such as lake size, mean depth, and primary productivity. We then parameterize Borgmann's (1987) biomass-size spectrum model and test the accuracy of its prediction of fish biomass in a wide range of lakes. Four versions of the model are tested and compared in each lake, two for planktonic communities and two for whole lake communities (plankton and benthos), which rely either on algal biomass or on invertebrate biomass as input data. The models developed by Thiebaux and Dickie (1992) unfortunately could not be tested because they require detailed size structure data, which were not available from the IBP studies.

Methods

Mean annual biomasses of phytoplankton, phytobenthos, zooplankton, zoobenthos, and fish were assembled for a worldwide set of lakes. We used data collected during the IBP (1965–1975). The goal of IBP-Freshwater was to estimate productivity of the world's limnetic systems, to quantify energy transfers through these whole systems, and to compare their energy budgets. In each system, groups of researchers intensively measured the biomass and productivity of planktonic and benthic organisms using similar sampling and processing techniques. Such complete and standardized data sets are rare and offer rigorous tests of biomass-size spectrum models for the prediction of fish biomass in lakes.

We compiled biomass data from the final IBP reports deposited in 1972–1973 at the Ferry House Laboratory of the Freshwater Biological Association on Lake Windermere, England, from subsequent publications, and directly from the researchers (Table 1). In cases of discrepancies between values reported in the IBP final reports and in later publications, we used the most recent version of the data, assuming that sample processing had been completed and (or) data had been revised.

Algae, invertebrates, and fish were sampled on different time schedules, reflecting their different life cycles. The links and transfers between groups of organisms that function at distinct time scales can be complex (Carpenter 1988; Denman et al. 1989), and at present there is no standardized method to decide on which time scale differ-

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Table 1. Sampling methods and protocol used to measure seasonal or annual mean biomass of different groups of organisms.

	Phytoplankton					Phytobenthos				
	Sampler	Units	n_{site}	n_{date}	n_{year}	Sampler	Units	n_{site}	n_{date}	n_{year}
Batorin[a]	?	V	?	?	3	nm	nm	nm	nm	nm
Boljshoy Harbey[b]	B	V	3–4	3–4	2	nm	nm	nm	nm	nm
Char	B	V	1	17–20	2	nm	nm	nm	nm	nm
Dusia	?	V	?	4	1	nm	nm	nm	nm	nm
Esrom	B	Ch	1	?	?	nm	nm	nm	nm	nm
Finstertalersee	B	V	1	15–18	2	C	V	23	9–15	2
George	B,T	Ch	1–20	33–35	2	C	Ch	1	1	1
Kiev[a]	?	V	?	6	1	?	E	?	?	1
Kinneret	B	V	1	52	2	nm	nm	nm	nm	nm
Kojima	?	Ch	1	2–7	5	nm	nm	nm	nm	nm
Myastro[a]	?	V	?	?	3	nm	nm	nm	nm	nm
Naroch[a]	?	V	?	?	3	nm	nm	nm	nm	nm
Neusiedlersee	?	V	3–20	?	2	?	V	3	13–15	2
Obelija	?	V	?	4	1	nm	nm	nm	nm	nm
Ovre Heimdalsvaten	?	V	2	14–16	2	nm	nm	nm	nm	
Port-Biehl[c]	B	V	1	6–9	4	G	D	10	4	1
Rybinsk[a]	?	V	6	7–11	10	nm	nm	nm	nm	nm
Suwa	B	Ch	1	11–17	3	nm	nm	nm	nm	nm
Tatsu-numa	P	Ch	3–7	3	1	G	C	?	2	1
Tjeukemeer	B	Ch	3	19–20	2	nm	nm	nm	nm	nm
Tsimliansk[a]	?	V	3	4–6	3	nm	nm	nm	nm	nm
Warniak[d]	B	V	2–3	6–28	3	nm	nm	nm	nm	nm
Wingra	B	D	1	12	2	nm	nm	nm	nm	nm
Yunoko	B	Ch	1	12	1	nm	nm	nm	nm	nm

Note: For sampling method (sampler), values in parentheses are mesh size (in μm or in mesh code). Units are those in which biomass was reported in the original study. The number of sites (n_{site}), dates (n_{dates}), and years (n_{year}) sampled are given. Sampling methods: A, artificial substrate; B, bottle; C, corer; E, electrofishing; FC, fisheries catch; G, grab; L, Leslie's catch ratio method; M, mark–recapture; N, net; P, pump; S, Schindler–Patalas trap; Se, seine; So, sonar; T, tube; Tr, transect. Units: C, carbon; Ch, chlorophyll; D, dry mass; E, energy (kcal; 1 kcal = 4.18 kJ); F, fresh mass; V, biovolume. Question marks indicate unknown values; nm, not measured. Data for the lakes and reservoirs were gathered from the IBP reports and more recent publications: Char: Roff and Carter (1972), Kalff et al. (1975), Rigler (1974, 1975, 1978); Esrom: Jónasson (1972, 1984), Jónasson et al. (1990); Finstertalersee: Bretschko (1975); George: Ganf (1972), Burgis et al. (1973), Burgis (1974), Gwahaba (1975), Vinier (1977); Kiev: Gak et al. (1972), Zeeb and Maistrenko (1972); Kinneret: Berman et al. (1992), Walline et al. (1992); Kojima: Adachi et al. (1975); Neusiedlersee: Löffler (1979); Øvre Heimdalsvaten: Lien (1978), Tangen and Brettum (1978); Port-Bielh: Capblancq (1972, 1973), Rey and Capblancq (1975), J. Capblancq, personal communication; Rybinsk: Sorokin (1972), Hrbáček (1984); Suwa: Sakamoto et al. (1975); Tatsu-numa: Abe et al. (1975); Tjeukemeer: Beattie et al. (1972), Goldspink and Banks (1975), Moed and Hoogveld (1982); Warniak: Hillbricht-Ilkowska and Węgleńska (1973), Kajak and Dusoge (1973), Spodniewska and Hillbricht-Ilkowska (1973), Zawisza and Ciepielewski (1973); Wingra: Prenti et al. (1977); Yunoko: Arai et al. (1975).

[a]Sampled during May–October.
[b]Sampled during July–August.
[c]Sampled during July–October.
[d]Sampled during April–October.

ent groups of organisms should be compared. We decided, therefore, to standardize the time frame of our analysis and to compare the biomass of different groups of organisms averaged over the same period, either seasonal or annual, depending on the data available. The sampling protocols used in each lake are summarized in Table 1. Instantaneous biomass measurements were averaged over all sampling stations, weighted for each sampling date (when sampling schedules were irregular), and averaged over all years sampled. To allow planktonic and benthic organisms to be compared, mean annual biomasses reported in volumetric units (grams per cubic metre) were multiplied by lake mean depth to obtain areal biomass values (grams per square metre). We converted all biomass values to grams fresh mass per square metre, the units most often reported (Table 1). Conversion factors reported by the authors were preferred, but standard conversion factors were used, when necessary, for phytoplankton (carbon:chlorophyll = 45:1, carbon : fresh mass = 0.12:1; Strickland 1960) and for zooplankton and zoobenthos (fresh mass : dry mass = 10:1; Winberg 1971). In 12 lakes (Table 1), fish biomass was estimated from fisheries catch (no correction) and should be considered with caution. We included these data in our analysis because there are few lakes where all major groups of organisms (algae, invertebrates, fish) have been sampled. We verified, nevertheless, that none of our conclusions were solely based on data from these lakes.

The biomass of organisms per logarithmic size-class (B_i) was calculated for each group of organisms i (phytoplankton, phytobenthos, zooplankton, zoobenthos, fish) as $B_i = B/N_c$, where B is the seasonal or annual mean biomass and N_c is the number of logarithmic size-classes covered by these organisms. N_c was calculated as $N_c = \log_{10}(M_{max}) - \log_{10}(M_{min})$, where M_{min} and M_{max} are the average body size of the smallest and of the largest species reported, respectively (in micrograms fresh mass). In lakes where M_{min} and M_{max} were not reported, we used the arithmetic average of N_c calculated at other sites.

The mean body size of each group of organisms i (M_i) was approximated as the geometric average of the smallest and largest species reported, as follows: $\log_{10}(M_i) = (\log_{10}(M_{min}) + \log_{10}(M_{max}))/2$. In lakes where M_{min} and M_{max} were not reported, we used the arithmetic average of M_i from other sites.

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Zooplankton					Zoobenthos					Fish	
Sampler	Units	n_{site}	n_{date}	n_{year}	Sampler	Units	n_{site}	n_{date}	n_{year}	Sampler	n_{year}
? (No. 70)	F	?	14–21	2	?	?	?	27	1	FC	1
?	F	?	?	2	?	F	5	4–6	2	FC	?
S (30)	D	5	34	1	C,A	C	?	1–4	1	M	4
?	F	?	?	1	?	F	?	?	1–3	FC	1
B (80)	F	1	~39	1	G (200)	D	?	4–25	1–5	FC	12
P (?)	F	1	15	1	C,G (100)	F	26	?	?	M	?
T (80)	D	20	9	1	C (333)	C	5	7	1	Se	1
N (No. 60)	F	?	6	1	G	F	?	1	1	FC	2
T,B (70)	F	3–5	?	3	nm	nm	nm	nm	nm	So	3
N (?)	D	12	3	1	G (?)	D	51	4	1	M	2
? (No. 70)	F	?	14–21	3	?	?	?	27	1	FC	1
? (No. 70)	F	?	14–21	3	?	?	?	27	1	FC	1
P,B,N (50)	D	4–16	12	2	C (100)	D	3	18	1	Se	2
?	F	?	?	1	?	F	?	?	1	FC	1
N (20–45)	F	4–8	12–15	3	nm	nm	nm	nm	nm	L	2
B (50)	D	2	17	1	G (280)	F	12–15	7	1	M	1
B	F	6	12	15	G (Nos. 17–23)	F	?	?	?	FC	23
N (XX17)	D	1	15–20	2	G (?)	F	1	6–8	2	FC	4
N (XX16)	D	1	6	1	? (10)	D	?	?	?	M	?
B (80,120)	F	10	>32	1	nm	nm	nm	nm	nm	FC	3
N (?)	F	?	?	3	nm	nm	nm	nm	nm	Tr	1
N (50)	F	2–3	6–28	3	C (400)	F	3	18	1	Se,E	3
B,N (158)	D	3	18–42	3	G (60)	D	6	?	1	M	3
N (XX13,XX17)	D	1	9	1	nm	nm	nm	nm	nm	FC	2

We tested how sensitive our conclusions are to missing M_{min} and M_{max} values. For each group where data were missing, we assigned random values of M_{min} and M_{max} (assuming a uniform distribution), within the range of observed minimum and maximum body sizes for the group. From this information, we then calculated the number of size-classes and mean body size, predicted fish biomass in the lake (see below), and compared these predictions with observed fish biomass by calculating the root mean square error (RMSE). We repeated this procedure 1000 times. Predictions of fish biomass from either phytoplankton or zooplankton biomass were always very different from observed fish biomass, regardless of the values of M_{min} and M_{max} used (RMSE range 18.7–34.3 g·m^{-2} for phytoplankton and 19.8–26.4 g·m^{-2} for zooplankton). Therefore, uncertainties about the N_c and the M_i of each group of organisms do not modify our conclusions.

Patterns in biomass-size spectra

The biomass of planktonic organisms found in logarithmic size-classes is generally found to decrease from phytoplankton to fish. Biomass-size spectra are hypothesized to have constant slopes with intercepts that vary systematically with lake characteristics (Boudreau and Dickie 1992) or to have slopes that vary systematically with lake characteristics (Sprules and Munawar 1986; Ahrens and Peters 1991). To test these hypotheses, we fitted the following model, using forward and backward stepwise multiple regression:

$$(1)\quad \log(B_i) = a + b(\log(M_i)) + c_1(\log(A)) + c_2(\log(\bar{z})) + c_3(\log(\mathrm{PP})) + d_1(\log(M_i \times \log(A)) + d_2(\log(M_i) \times \log(\bar{z})) + d_3(\log(M_i) \times \log(\mathrm{PP}))$$

where B_i is biomass per logarithmic size-class of component i (phytoplankton, zooplankton, or fish), M_i is mean body size of component i, A is lake surface area (in square kilometres), $\bar{z}$ is lake mean depth (in metres), PP is primary productivity (in grams carbon per square metre per period), and a, b, c, and d are fitted coefficients. Significant coefficients associated with $\log(M_i)$ (b, d_1–d_3) suggest that the biomass of organisms found in a logarithmic size-class is related to the mean body size of these organisms. We expect biomass-size spectra with negative slopes (negative b coefficient). Significant coefficients

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for interaction terms suggest that the slopes of community size spectra vary systematically with lake surface area (d_1), lake mean depth (d_2), or primary production (d_3). Significant coefficients associated with environmental variables (c_1–c_3) suggest that biomass per size-class varies systematically with each variable, irrespective of mean body size. All analyses use common (i.e., base 10) logarithms and a significance level of 0.05.

Prediction of fish biomass using Borgmann's model

Uwe Borgmann (1987) developed a model to predict fish biomass from allometry. In this model, regularities in community size distributions and in energy transfers among size-classes are used to predict the biomass or production of a size-class from any other component of the community. Borgmann's model is based on the premise that the rate of production (P) and the rate of biomass turnover (P/B; production to biomass ratio) of an organism are both allometric functions of M. The biomass of any group of organisms ranging in body size from x to y (B_{xy}; e.g., fish biomass) can then be calculated from

$$(2) \quad B_{xy} = b(M_{max}^{n-\varepsilon} - M_{min}^{n-\varepsilon}),$$

where M_{min} and M_{max} are the smallest and largest body sizes being considered (e.g., the smallest and largest fish) and b, n, and ε are coefficients empirically determined from the biomass and production of other, more easily sampled, components of the community (e.g., phytoplankton or zooplankton). The exponent of the allometric relationship for mass-specific rates of production (n) can be calculated as follows:

$$(3) \quad P/B = aM^{-n}$$

where M is mean body mass (Borgmann 1987). The value of n is assumed to be the same for all groups of organisms and can theoretically be estimated from any group of organisms (phytoplankton, phytobenthos, zooplankton, zoobenthos, or fish). Epsilon (ε) is the particle size conversion efficiency, which measures the efficiency of biomass conversion from one size-class to another (Borgmann 1982). It is estimated iteratively from P and the range of M_{min} to M_{max} of two groups of organisms i and j, for example phytoplankton and zooplankton:

$$(4) \quad \frac{P_i}{(M_{max\,i}^{\varepsilon} - M_{min\,i}^{\varepsilon})} = \frac{P_j}{(M_{max\,j}^{\varepsilon} - M_{min\,j}^{\varepsilon})}$$

To estimate ε from eq. 4, we calculate P_i using values of ε in increments of 0.001, until the difference between calculated and measured P_i is minimized. Large ε values indicate low conversion efficiencies. From these values of n and ε, coefficient b is then calculated using eq. 2 (e.g., Borgmann 1985).

We used the detailed data for phytoplankton and zooplankton from the IBP to estimate n, ε, and b in as many lakes as possible and used these coefficients to predict fish biomass in a wide range of lakes. We compared these predictions with measured fish biomass, both in absolute and in relative terms. Absolute discrepancies between observed and predicted fish biomass (the residuals) were used to judge the predictive value of biomass-size spectrum models. Relative discrepancies, measured as $\log_{10}$(observed/predicted), are perhaps less important than the absolute values but are more appropriate in assessing biases in the models. All analyses were performed in SAS for microcomputers, version 6.08 (SAS Institute Inc., Cary, N.C.).

Results and discussion

The IBP-Freshwater program provided biomass measurements for phytoplankton, zooplankton, fish, and, in a subset of the studies, phytobenthos and zoobenthos from 24 lakes and reservoirs distributed worldwide (Table 2). The lakes and reservoirs ranged almost 6 orders of magnitude in surface area (0.008–4550 km^2), 20-fold in mean depth (1.3–25 m), and 280-fold in primary productivity (5–1403 g C·m^{-2}·year^{-1}). Anthropogenic impact also varied from pristine arctic or alpine lakes (e.g., lakes Boljshoy Harbey, Char, Port-Biehl, and Finstertalersee) to managed lakes (e.g., commercial fisheries in lakes George, Kinneret, and Suwa; fish stocking in Lake Warniak), to reservoirs built and heavily controlled by humans (e.g., Tjeukemeer, Kojima, and Kiev reservoirs). Because these systems varied widely in their characteristics, they allowed an assessment of the range of lakes over which models involving biomass-size spectra can be used to predict fish biomass.

The mean annual biomass of different groups of organisms varied by more than two orders of magnitude among lakes (Table 2). Phytoplankton biomass ranged from 0.6 to 200 g·m^{-2} fresh mass (333-fold), zooplankton biomass from 0.2 to 29 g·m^{-2} (145-fold), and fish biomass from 0.3 to 111 g·m^{-2} (370-fold). Benthic organisms were sampled at fewer sites, but covered similar biomass ranges: benthic algae from 2 to 422 g·m^{-2} (211-fold) and benthic invertebrates from 1.2 to 139 g·m^{-2} (116-fold). The biomass of each of these groups was spread over species that range several orders of magnitude in body size. Phytoplankton species ranged in mean body size from 0.1 μm^3 (*Aphanocapsa delicatissima* in Lake Wingra) to 8.4×10^5 μm^3 (*Melosira islandica* in Lake Warniak), and covered, on average, 3.9 logarithmic size-classes in any given lake (range from nine lakes: 2.3–5.7). Zooplankton (rotifers and crustaceans) and fish species covered, on average, 2.8 (range from 9 lakes: 1.3–3.7) and 2.3 (range from 12 lakes: 0.8–4.5) logarithmic size-classes, respectively. Annual mean B_i also varied by more than two orders of magnitude among sites: 0.15–51 g·m^{-2} fresh mass for phytoplankton, 0.2–23 g·m^{-2} for zooplankton, and 0.07–63 g·m^{-2} for fish.

The mean body size for each group of organisms was calculated as the geometric mean of the smallest and the largest species found in each lake. Mean phytoplankton body size ranges from 72 to 3549 μm^3 in different lakes (n = 9 lakes), with an average of 823 μm^3 (10^{-3} μg fresh mass). Mean zooplankton (rotifers and crustaceans) body size ranged from 1.7 to 7.1 μg fresh mass (n = 9 lakes) with an average of 4.0 μg, and mean fish size ranged from 0.2 to 790 g fresh mass (n = 12 lakes) with an average of 137 g (10^8 μg).

Biomass-size spectra of planktonic communities

Biomass-size spectra in each lake were based on the seasonal or annual mean biomass of three groups of organisms (phytoplankton, zooplankton, fish). The slopes of linear relationships fit by simple regression analysis in each lake varied from –0.14 to 0.14, or, if lakes with fisheries catch data were excluded, from –0.08 to 0.14. These slopes suggest that fish have lower, similar, or higher biomasses per logarithmic size-class relative to phytoplankton. Statistical tests of the significance of these slopes and of differences in slopes among lakes, however, were very weak because of the small number of data points per lake (n = 3). If more detailed data were available (which was not the case), we could have increased the number of points by subdividing the groups we have defined here (phytoplankton, zooplankton, fish) into several size-classes. At a finer scale, biomass-size spectra are shaped as successive domes instead of straight lines (Boudreau et al. 1991), and within each of these domes, there is evidence that the biomass or density of organisms in different size-classes is highly correlated. For

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Table 2. Morphometry, phytoplankton productivity, and biomass (all as fresh mass) of different groups of organisms in lakes of increasing productivity.

Lake	Area (km^2)	Mean depth (m)	Phytoplankton production ($g\ C \cdot m^{-2} \cdot year^{-1}$)	Phytoplankton biomass ($g \cdot m^{-2}$)	Phytobenthos biomass ($g \cdot m^{-2}$)	Zooplankton biomass ($g \cdot m^{-2}$)	Zoobenthos biomass ($g \cdot m^{-2}$)	Fish biomass ($g \cdot m^{-2}$)
Char, Canada	0.5	10.2	5	2.5	na[a]	0.2	11.2	1.0
Tatsu-numa, Japan	0.008	3.7	12	0.6	11.7[b]	0.2	4.8	4.8
Boljshoy Harbey, Russia	21.3	4.6	15	1.6	na	1.7	4.7	0.6[c]
Ovre Heimdalsvatn, Norway	0.8	4.7	17	0.8	na	1.3	6.0[b]	1.7
Port-Biehl, France	0.2	9.0	23	1.5	422.0	8.5	3.0[d]	3.6
Finstertalersee, Austria	0.2	14.8	27	7.3	7.8	0.5	2.0	2.3
Dusia, Lithuania	23.2	14.6	35	15.8	na	7.1	11–43.0[d]	7.5[c]
Obelija, Lithuania	5.7	4.5	41	10.5	na	9.6	2–39.0[d]	6.0[c]
Naroch, Bielorussia	80.1	9.0	57	7.2	na	9.9	5.7	7.4[c]
Rybinsk, Russia	4550.0	5.6	64	10.6	na	2.6	3.2[b]	0.7[c]
Neusiedlersee, Austria	300.0	1.3	79	0.8	1.8	1.7	1.2	0.3
Yunoko, Japan	0.4	7.3	107	84.1	na	15.6	1.5[b]	13.0[c]
Warniak, Poland	0.4	1.5	167	4.6	na	4.4	9.7	8.5
Myastro, Bielorussia	13.1	5.4	168	25.9	na	17.2	2.4	5.7[c]
Kiev, Ukrainia	992.0	4.0	178	38.3	62.0	21.0	139.0	0.8[c]
Esrom, Denmark	17.3	13.5	190	24.2	na	23.0	112.0	2.4[c]
Batorin, Bielorussia	6.2	3.0	192	54.6	na	10.8	2.5	2.9[c]
Wingra, Wisconsin, U.S.A.	1.4	2.4	320	10.7	na	29.2	3.6[c]	54.6
Tjeukemeer, Netherlands	21.5	1.5	371	48.4	na	3.9	na	1.1[c]
Suwa, Japan	14.4	4.1	439	65.7	na	16.3	51.8	28.9[c]
Tsimliansk, Russia	2702.0	8.8	439	31.9	na	12.0	na	17.2
Kojima, Japan	10.7	1.6	474	70.8	na	15.7	2.2	110.9
Kinneret, Israel	167.8	25.0	619	74.0	na	19.3	na	16.6
George, Uganda	250.0	2.4	1403	199.6	259.0[b]	8.9	10.4	29.0

[a]na, not available
[b]Estimated by authors.
[c]Insects only.
[d]Maximum (summer sample).
[e]Fisheries catch.

example, zooplankton size distributions have two peaks of density (or biomass) and the sizes of these peaks vary independently of one another among lakes. However, within each peak, densities of different size-classes are correlated (Cyr and Pace 1993). A similar pattern has been found in benthic invertebrate communities (Rasmussen 1993), and may be expected in other groups of organisms. Narrowing size-classes, therefore, would not necessarily provide more information about the primary structure of biomass-size spectra. We assume that our approach, based on three points per lake, captured the overall structure of biomass-size spectra.

Biomass-size spectra of planktonic communities were compared at a large scale along gradients of lake characteristics. Relationships between the slopes and intercepts of biomass-size spectra and lake surface area, lake mean depth, and primary productivity were tested using eq. 1 (see Methods). The best equation describing this relationship is

$$(5)\quad \log(B_i) = 0.8\ (\pm 0.1)\log(\mathrm{PP}) + 0.4\ (\pm 0.1)\log(\bar{z}) - 0.03\ (\pm 0.006)\log(M_i) \times \log(\mathrm{A}) - 1.4\ (\pm 0.2)$$

$R^2 = 0.60$, residual mean square = 0.18, $p < 0.001$, $n = 72$,

where B_i is the biomass of a group of organisms i per logarithmic size-class (in grams fresh mass per square metre), PP is annual primary production (in grams carbon per square metre per year), is lake mean depth (in metres), M_i is mean body size of a group of organisms i (in micrograms fresh mass), and A is lake surface area (in square kilometres).

The overall slope of biomass-size spectra in lakes was not significantly different from zero (coefficient of $\log(M_i)$ in eq. 1, $p > 0.1$). A similar result is found when we exclude lakes with fisheries catch data from the analysis. This unexpected result reflects the inconsistent direction of the slopes of biomass-size spectra in individual lakes when we consider only planktonic organisms ($b = -0.14$ to 0.14). These results suggest that the importance of benthic organisms and of allochtonous inputs as additional food sources may vary widely among lakes.

The biomass of planktonic organisms per logarithmic size class (B_i) is positively related to primary productivity ($p(t) < 0.001$). The coefficient associated with log(PP) suggests that the B_i values of phytoplankton, zooplankton, and fish increase, on average, 7-fold with every 10-fold increase in primary productivity (Fig. 1). The intercepts of biomass-size spectra, therefore, increase with primary productivity. Unlike Sprules and Munawar (1986) and Ahrens and Peters (1991), however, we found no evidence that the slopes of biomass-size spectra vary systematically with lake productivity (i.e., the interaction term $\log(M_i) \times \log(\mathrm{PP})$ was not significantly different from zero, $p > 0.1$). Both previous studies compared detailed size spectra ranging from bacteria to large invertebrates and found

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Fig. 1. Relationship between the biomass (fresh mass) per logarithmic size-class of different groups of organisms (B_i) and annual primary production. Circles, phytoplankton; triangles, zooplankton; squares, fish.

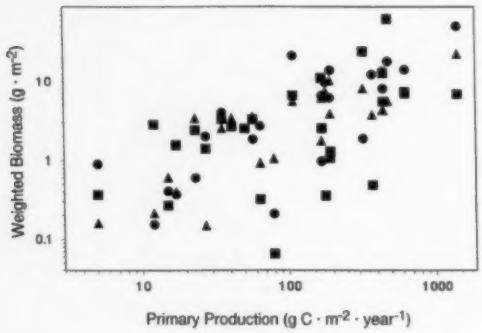
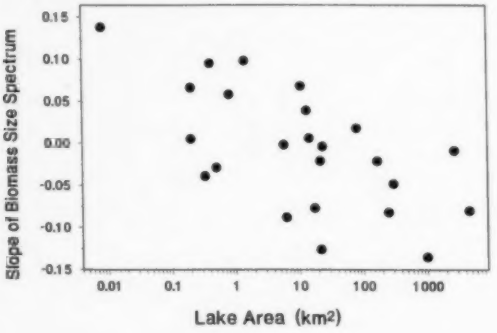


Fig. 2. Relationship between the slope of biomass-size spectra in different lakes and lake surface area.

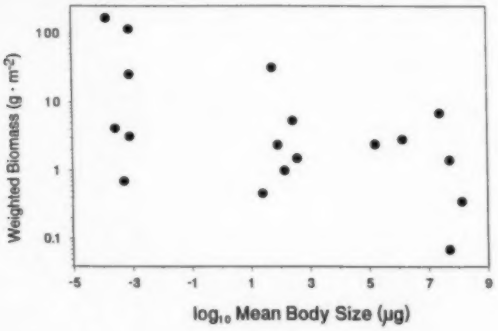


significant, but weak, positive relationships between the slopes of size spectra and total phosphorus concentration, a measure of lake productivity (Sprules and Munawar 1986, Fig. 4; r^2 = 0.28 in Ahrens and Peters 1991). Our study is based on coarser size-classes but extends the analysis to fish. In lakes covering more than two orders of magnitude in productivity, we found that the intercepts of biomass-size spectra vary systematically with annual primary production, but not their slopes.

B_i is also positively related to lake mean depth ($p(t)$ = 0.015). This relationship, however, varies with the units chosen to express B_i. Biomass expressed in volumetric units (grams fresh mass per cubic metre), instead of areal units, decreases with increasing mean depth ($p(t) < 0.001$). These relationships probably reflect the uneven vertical distribution of organisms in the water column, with most of the production usually occurring in surface waters.

The interaction term ($\log(M_i) \times \log(A)$) suggests that the relationship between B_i and the size of the organisms differs in small and in large lakes. Fish tend to reach higher biomass than phytoplankton and (or) zooplankton in small lakes but lower biomass in large lakes (Fig. 2). Over the range of lake sizes covered in our analysis (0.008–4550 km^2), fish biomass ranged from 4-fold higher to 11-fold lower than the biomass of phytoplankton. In our data set, however, fish biomass was measured directly in many small lakes but was estimated from

Fig. 3. Biomass-size spectra of whole lake communities (including plankton and benthos). Data are from six lakes (see Table 2). Biomass and mean body size are expressed as fresh mass.



fisheries catches in most large lakes and reservoirs. To test whether the interaction term could result from a methodological bias, we repeated our analysis excluding all lakes where commercial catches were used as estimates of fish biomass (Table 1). The new equation, based on the remaining 12 lakes, also includes a significant interaction term with lake surface area (n = 36, $p(t)$ = 0.001), suggesting no strong influence of this methodological bias on our results. The slopes of biomass-size spectra become more negative with increasing lake size.

In summary, biomass-size spectra of planktonic communities vary among lakes. Their intercepts are positively related to primary productivity and to lake mean depth. Their slopes become more negative with increasing lake size.

Biomass-size spectra of whole lake communities

Planktonic organisms are usually not independent from the benthos in lakes, but these interactions are rarely considered in biomass-size spectrum models. Boudreau and Dickie (1992) compared biomass-size spectra of phytoplankton, zooplankton, zoobenthos, and fish from seven marine and freshwater sites and found that their slopes were constant (–0.04 to –0.07) whereas their intercepts appeared to increase with productivity of the site. These results suggest the existence of a consistent general structure among aquatic communities. To examine this possibility in the IBP lakes, we repeated our analysis (eq. 1) for the six lakes where both planktonic and benthic biomass, including benthic algae, were measured (Table 2). These lakes cover ranges of surface area (0.008–4550 km^2), mean depth (1.3–14.8 m), and annual primary production (12–1403 g C·m^{-2}·year^{-1}) similar to those of the full data set. When both planktonic and benthic organisms are considered, B_i decreases with increasing M_i as

$$\log(B_i) = -0.11\ (\pm 0.04) \log(M_i) + 0.7\ (\pm 0.2),$$
$$r^2 = 0.30, \text{ residual mean square} = 0.56, P = 0.02, n = 18$$

(Fig. 3). Biomass per size-class generally decreased across the size spectrum, but we found no evidence that the large unexplained variance around this relationship could be attributed to simple lake characteristics. The slopes and intercepts of biomass-size spectra were not significantly related to lake surface area, mean depth, or phytoplankton productivity ($p > 0.1$). The size structure of lacustrine communities was more variable than found by Boudreau and Dickie (1992).

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Table 3. Coefficients of particle-size-conversion efficiency (ε) calculated from different groups of organisms: ε_{A-I}, algae and invertebrates; ε_{A-F}, algae and fish; ε_{I-F}, invertebrates and fish; ε_{A-I-F}, algae, invertebrates, and fish.

Lake	ε_{A-I}	ε_{A-F}	ε_{I-F}	ε_{A-I-F}
Plankton only				
Char	0.23	0.23	0.23	0.23
Ovre Heimdalsvatn	0.28	0.12	0.04	0.12
Port-Bielh	0.23	na[a]	na	na
Tjeukemeer	0.17	na	na	na
Warniak	0.19	na	na	na
Mean	0.22	0.19[b]	0.17[a]	
Plankton and benthos				
Char	0.22	0.31	0.36	0.26
Ovre Heimdalsvatn	0.24	0.14	0.08	0.14
Mean	0.23	0.22	0.22	0.20

[a]na, not available.

[b]Averages over five lakes. Missing values of ε_{A-F} and ε_{Z-F} are replaced by ε_{A-I} measured in the same lake.

Parameterization of Borgmann's biomass-size spectrum model

To predict fish biomass using Borgmann's model, one must measure or select values for three parameters, b, n, and ε (see Methods). These parameters adjust the model for differences in the intercepts and the slopes of biomass-size spectra. Differences in intercepts are routinely corrected in each lake where the model is applied, by calculating coefficient b from the input data (e.g., phytoplankton or zooplankton biomass; eq. 2). Differences in slope, on the other hand, are adjusted using two coefficients (n and ε) about which very little is known. These coefficients are both estimated from the biomass and the production of at least two groups of organisms of different sizes (e.g., phytoplankton and fish, zooplankton and fish; eqs. 3 and 4). Because such data are rarely available, the few measured values of n and ε have been used in all other models (e.g., Borgmann 1983; Borgmann et al. 1984; Leach et al. 1987; Sprules et al. 1991). The values of n were originally measured in Lake Ontario (Borgmann 1985; Minns et al. 1987) and in the Sargasso Sea (Sheldon et al. 1977) and range between 0.16 and 0.24. These measurements are close to the general value of 0.15–0.17 found for phytoplankton (Tang 1995; $n = 68$), for planktonic and benthic invertebrates (Plante and Downing 1989; $n = 137$), and for fish (Downing and Plante 1993, $n = 100$). The available measures of ε are from Lake Ontario (Minns et al. 1987, $\varepsilon = 0.20$; Borgmann and Whittle 1994, $\varepsilon = 0.28$–0.33); it is not known how they may vary among lakes.

Even in the detailed data from the IBP, there are few lakes for which total biomass and total production have been measured in two groups of organisms. Production data necessary to estimate ε coefficients were available from five lakes for planktonic organisms and from two lakes for combined planktonic and benthic organisms (Table 3). The coefficients measured from phytoplankton and zooplankton production data (ε_{A-I}) ranged from 0.17 to 0.28 (Table 3) and were close to the values reported from Lake Ontario (Minns et al. 1987; Borgmann and Whittle 1994). Similar values were obtained when benthic organisms were also included in the analysis (Table 3). The lowest ε coefficients, indicating high transfer efficiencies, were measured in two shallow, heavily managed eutrophic lakes (Tjeukemeer, Lake Warniak), while higher ε values and lower transfer efficiencies were found in the three pristine oligotrophic lakes (Char Lake, Øvre Heimdalsvatn, Lake Port-Biehl).

Borgmann's biomass-size spectrum model (1987) assumes that ε is constant from the smallest to the largest organisms being considered. Contrasting results from two lakes in the IBP data set suggest that this assumption may not be appropriate in all lakes (Table 3). In Char Lake, $\varepsilon = 0.23$ for all groups of organisms. In Øvre Heimdalsvatn, however, ε ranged from 0.04 for zooplankton and fish to 0.28 for phytoplankton and zooplankton. Similar differences were found when considering planktonic and benthic organisms simultaneously (Table 3). The ε values varied up to seven fold among groups of organisms, resulting in differences of up to two orders of magnitude in predicted fish biomass. Values for ε must be estimated or selected very carefully.

Prediction of fish biomass

We used Borgmann's model to predict fish biomass in each lake, both from the biomass of phytoplankton and from the biomass of zooplankton. Equation 2 was fitted with $n = 0.16$ (Plante and Downing 1989; Downing and Plante 1993; Tang 1995) and with measured or average ε values (ε_{A-F}) to predict fish biomass from algal biomass and ε_{I-F} to predict fish biomass from invertebrate biomass (Table 3). Because we used the average values of ε ($\varepsilon_{A-F} = 0.19$ or $\varepsilon_{I-F} = 0.17$) in all but two lakes, the coefficient $n - \varepsilon$ ranged mostly between −0.01 and −0.03, a value similar to that used in other studies (e.g., Minns et al. 1987; Sprules et al. 1991). Higher values of ε (e.g., Table 3) would result in predictions of lower fish biomass. Values of b were calculated from B_{xy}, M_{min}, and M_{max} of phytoplankton and of zooplankton using eq. 2. These values of n, ε, and b, along with the M_{min} and M_{max} of fish, were then used in eq. 2 to predict fish biomass.

Fish biomasses predicted from phytoplankton (Fig. 4A) and zooplankton (Fig. 4B) ranged from 0.1 to 99.4 $g \cdot m^{-2}$ fresh mass and were only weakly correlated to measured fish biomasses ($r^2 = 0.19$, $p = 0.03$ in both cases). The absolute difference between measured fish biomass and predicted fish biomass (|observed − predicted|, the residual) exceeded 5 $g \cdot m^{-2}$ in almost half of the lakes (Fig. 5A), while predicted fish biomass was within three fold of observed biomass (±0.5 logarithm units) in only half of the lakes (Fig. 5B). Fish biomass was overestimated by as much as 70 $g \cdot m^{-2}$ fresh mass in Lake George (Fig. 4A) and underestimated by as much as 103 $g \cdot m^{-2}$ in Lake Kojima (Fig. 4B). The predictions may represent as little as 3% of the observed fish biomass (Tatsunuma, Fig. 4A) or as much as 1800% (Tjeukemeer in Fig. 4A).

Most lakes where fish biomasses were overestimated by Borgmann's model (points below the 1:1 line in Fig. 4) were lakes where fish biomass was "measured" from fisheries catches (Fig. 4, crosses). These catches represent only a portion of the fish present in a lake and provide low estimates of fish biomass. It may not be surprising, therefore, that Borgmann's model overestimates fish biomass in many of these lakes. In lakes where better measurements of fish biomass are available, Borgmann's model tends to underestimate fish biomass (Fig. 4, points above the 1:1 line). In about half of these lakes fish biomass is predicted well (Fig. 4, four

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Fig. 4. Comparisons of observed fish biomass (as fresh mass) with predictions from Borgmann's biomass-size spectrum models. The models were developed for planktonic communities and the predictions are made from (A) phytoplankton biomass or (B) zooplankton biomass. The 1:1 line indicates perfect agreement between predictions and observations. Solid symbols represent lakes where fish biomass was measured directly (●, mark–recapture; ▲, other sampling methods listed in Table 1). Crosses represent lakes where commercial fisheries catch were used as a measure of fish biomass.

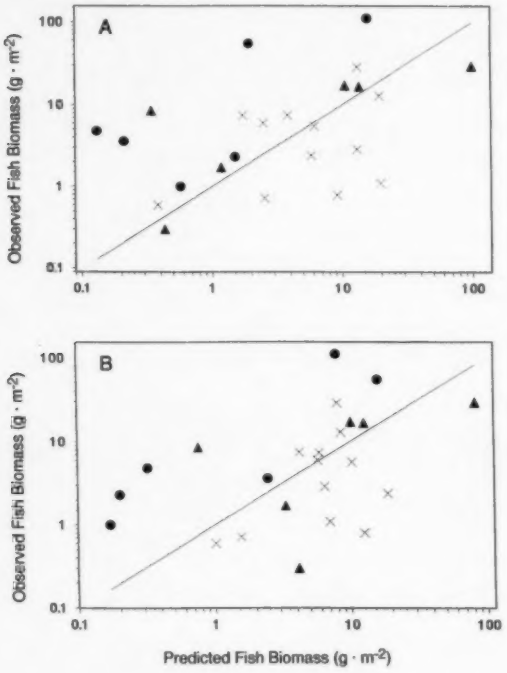
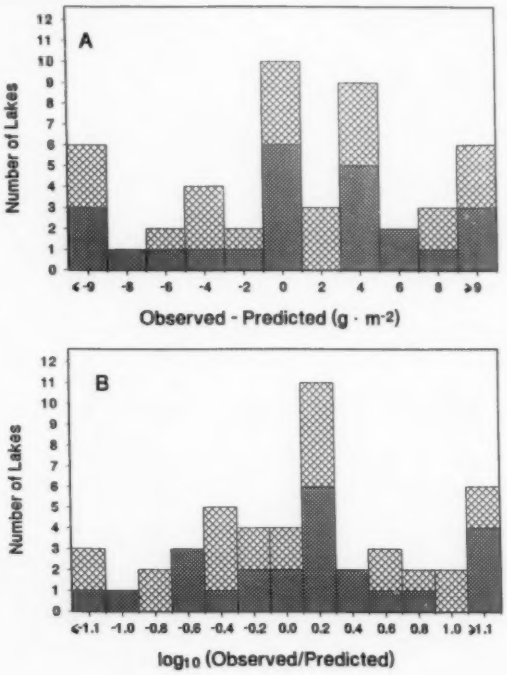


Fig. 5. Frequency distributions of (A) absolute and (B) relative discrepancies between predicted and observed fish biomass (fresh mass) in 24 lakes. Predictions made from phytoplankton biomass (fine grid) and from zooplankton biomass (coarse grid) are combined in each panel.



to six points close to the 1:1 line), but in other lakes, fish biomass is grossly underestimated or overestimated (Fig. 4). Predictions of fish biomass from phytoplankton biomass (Fig. 4A, solid symbols only; $r^2 = 0.41$, RMSE = 0.61, $p = 0.03$, $n = 12$) are more closely correlated to observed fish biomass than predictions from zooplankton biomass (Fig. 4B; $r^2 = 0.33$, RMSE = 0.65, $p = 0.05$, $n = 12$), suggesting that phytoplankton are a slightly better predictor of fish biomass than zooplankton.

Discrepancies, in relative terms, between predicted and observed fish biomass can be used to evaluate biases in the size spectrum models (Figs. 6D–6F). Fish biomass tends to be underestimated in small and in oligotrophic lakes and overestimated in large lakes (Figs. 6D–6F). This is consistent with suggestions that food sources other than phytoplankton (benthic algae, allochtonous carbon) are most important in small oligotrophic lakes (del Giorgio and Peters 1994). Bacterial and heterotrophic protozoan biomass are not included in our analyses because they were either not considered or not measured reliably in the 1960s. Our analysis, therefore, ignores any contribution of the microbial loop to the ecosystem, but this is consistent with other biomass-size spectrum models (e.g., Minns et al. 1987; Sprules et al. 1991; Boudreau and Dickie 1992). The omission of heterotrophic protozoans may result in underestimations of the food base available to invertebrates (zooplankton and zoobenthos) and, consequently, in low predictions of fish biomass. The underestimation of fish biomass in small lakes (Fig. 6D) may also suggest that benthic invertebrates, which are not considered here, contribute an important source of food for fish. The poorest predictions, in absolute terms (i.e., large residuals), are made in shallow eutrophic lakes (Figs. 6A–6C).

To test the effectiveness of including benthic organisms in biomass-size spectrum models to predict fish biomass, we repeated our analysis with combined planktonic and benthic data. The biomass of benthic algae was measured in 6 lakes and the biomass of benthic invertebrates was available from 21 lakes (Table 2). Given the benthic data, size spectrum models predicted fish biomass to range from 0.3 to 112 $g \cdot m^{-2}$, but these predictions were not related significantly to observed fish biomass (Fig. 7A, $p = 0.16$; Fig. 7B, $p = 0.7$). Fish biomass was overestimated by as much as 18 $g \cdot m^{-2}$ by algal data (Fig. 7A) and underestimated by as much as 83 $g \cdot m^{-2}$ by invertebrate data (Fig. 7B) in Lake George. In relative terms, the fish biomass predicted from combined benthic and planktonic organisms was as little as 3% (Kiev, Fig. 7B) or as much as 160% (Finstertalersee, Fig. 7A) of observed fish biomass. Moreover, the RMSEs (calculated from untransformed observed and predicted values) were not substantially lower for

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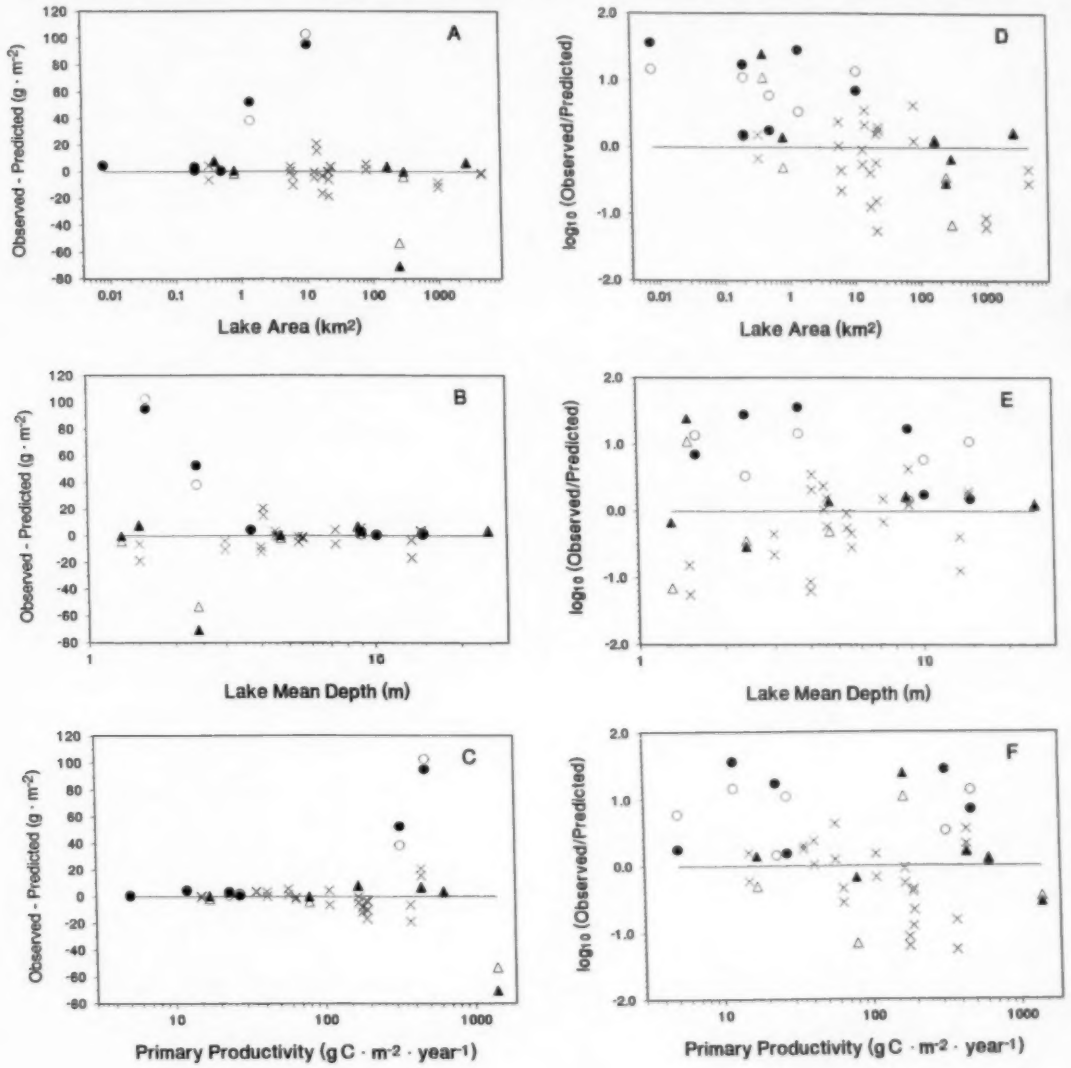
Fig. 6. Relationships between the accuracy of fish biomass predictions and lake characteristics. (A–C) Absolute and (D–F) relative discrepencies between observed and predicted fish biomass. Different symbols are used for different methods of measuring fish biomass: circles for mark–recapture methods, triangles for other sampling methods (see Table 1), and crosses for fisheries catches. Solid and open symbols represent predictions made from phytoplankton and zooplankton biomass, respectively. Biomass is expressed as fresh mass.



models of whole lake communities than for models of planktonic communities (predictions based on algal data: $RMSE_{plankton+benthos} = 6.83$ vs. $RMSE_{plankton} = 2.6$, $n = 6$; predictions based on invertebrate data: $RMSE_{plankton+benthos} = 29.2$ vs. $RMSE_{plankton} = 30.7$, $n = 15$). Predictions of fish biomass were, therefore, not improved by including benthic organisms in the biomass-size spectrum models. Moreover, methodological difficulties in sampling and measuring the biomass of benthic algae and invertebrates would limit their use in predicting fish biomass.

The slopes and the intercepts of biomass-size spectra vary systematically with lake characteristics. Despite these regularities, much variability in the structure and patterns of energy transfer of lacustrine communities remains unexplained. Fish biomass per logarithmic size-class may be lower than, similar to, or higher than phytoplankton biomass per logarithmic size-class. At this point, our ability to predict the biomass of one group of organisms from another using Borgmann's model is limited. More detailed biomass-size spectrum models (Thiebaux and Dickie 1992) could not be tested but would also need to allow for variability in predator–prey relationships between different groups of organisms, in different types of habitat, and at different sites. The application of biomass-size spectrum models to lakes will depend on a better knowledge of the

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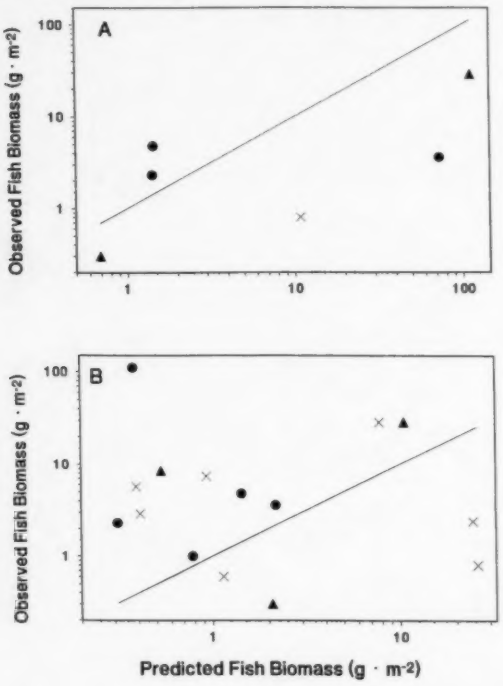
Fig. 7. Comparisons of observed fish biomass (as fresh mass) with predictions from biomass-size spectrum models. The models were developed for whole lake communities (plankton and benthos) and the predictions are made from (A) phytoplankton biomass or (B) zooplankton biomass. The 1:1 line indicates perfect agreement between predictions and observations. Symbols are as in Fig. 4.



variability in energy transfers between body size-classes (Borgmann's models) and between predators and prey (Thiebaux and Dickie's models) in lakes, especially in environments with complex physical structures (e.g., macrophytes, shoreline features). This is not an easy task.

Acknowledgments

We thank the members of the Freshwater Biological Association's Windermere Laboratory for their generosity and hospitality during our work on the IBP data; J. Capblancq, H. Kling and the staff of the Limnology Center of the University of Wisconsin for providing unpublished data; and P. Jónasson for references. A. Tinker helped to sort and organize the data. This research was funded by the Natural Sciences and Engineering Research Council of Canada and the ministère de l'Éducation du Québec (Fonds FCAR). The Groupe de recherche interuniversitaire en limnologie et en environnement aquatique and the Fonds FCAR provided postdoctoral fellowships for H.C.

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A two-step approach to estimating selectivity and fishing power of research gill nets used in Greenland waters

Holger Hovgård

Abstract: Catches of Atlantic cod (*Gadus morhua*) from Greenland gill-net surveys were analyzed by a two-step approach. In the initial step the form of the selection curve was identified as binormal, which was caused by fish being gilled or caught by the maxillae. Both capture processes could be described by normal distributions and could be related to mesh size in accordance with the principle of geometrical similarity. In the second step the selection parameters were estimated by a nonlinear least squares fit. The model also estimated the relative efficiency of the two capture processes and the fishing powers of different netting sections. Gilling was about three times as efficient as maxillae catching and fishing power could be related to the ratio of twine diameter to mesh size. It is proposed that information on how fish are caught should be included when modelling gill-net selectivity, as lack of such data may lead to the formulation of unrealistic selection models.

Résumé : Les prises de morue franche (*Gadus morhua*) lors de la campagne d'échantillonnage au filet maillant dans les eaux du Groenland ont été analysées par une approche à deux étapes. Dans la première étape, la forme de la courbe de sélection était binormale, ce qui était attribuable au poisson capturé par les ouïes ou par les maxillaires. Les deux procédés de capture pourraient être décrits par des distributions normales et pourraient être liés au maillage conformément au principe de similarité géométrique. Dans la deuxième étape, les paramètres de sélection ont été évalués au moyen d'un ajustement par les moindres carrés non linéaire. Le modèle a également estimé l'efficacité relative des deux méthodes de capture et du pouvoir pêchant des différentes sections du filet. La prise par les ouïes était environ trois fois plus efficace que le prélèvement par les maxillaires et le pouvoir pêchant pouvait être lié au rapport entre le diamètre du fil et la taille de la maille. On propose que cette information sur la façon de capturer les poissons soit incluse dans la modélisation de la sélectivité du filet maillant étant donné que l'absence de ces données peut entraîner la formulation de modèles de sélection peu réalistes.
[Traduit par la Rédaction]

Introduction

Gill nets are commonly used in studies to estimate the abundance and size structure of fish populations. Gill nets are, however, highly size selective (Hamley 1975). Therefore, it is necessary to use several mesh sizes simultaneously and to correct the observed size frequencies for the effects of mesh-size selectivity to estimate the size composition in a population.

There are several approaches available for estimating gill-net selectivity (Hamley 1975). Indirect methods, i.e., methods that do not require a knowledge of the size distribution in the population, are by far the most frequently used. These methods, which all require catch at size information from several different mesh sizes fished simultaneously, rely on two basic assumptions: (*i*) the principle of geometrical similarity (Baranov 1948), which states that selection depends only on the relative geometry of the fish to that of the mesh, and (*ii*) fishes of a given size are equally available to all mesh sizes used. Indirect methods do not utilize information on the processes by which the fish are caught, although in some cases the catch data are restricted to gilled fish only (e.g., Helser et al. 1991). Most often the selection curves take the form of a right-skewed distribution. The curve may be drawn by hand (Gulland and Harding 1961) or fitted to a theoretical distribution. Typically a lognormal distribution (McCombie and Fry 1960), a skewed normal distribution (Regier and Robson 1966), or a gamma distribution (Henderson and Wong 1991) have been used.

In the present study, the sizes of fish caught in different meshes could not be described by a unimodal selection curve. Each mesh caught two distinct size distributions of cod representing different capture processes (Hovgård 1988). It was also evident that the catch rates differed between mesh sections.

The purpose of this paper is to show that two capture processes (gilling and maxillae enmeshing) are of importance for the gill-net selection of cod and that the fishing power can be very different between net sections. The estimation procedure presented here contains two steps. First, the form of the selection curve was identified by scrutinizing the length distribution of the catches. This pattern was then used in modelling selection. The selection curve and the fishing power of the different net sections were estimated by a nonlinear least squares fit.

Materials and methods

Fishing gear and operational procedures

The data were obtained from annual gill-net surveys carried out in the fjords and coastal areas of western Greenland in July from 1985 to 1993. Because of the vast size of western Greenland the surveys were restricted to three inshore areas: Qarqortoq–Julianehåb (~60°N), Nuuk–Godthåb (~64°N), and Sisimiut–Holsteinsborg (~67°N).

Received May 31, 1994. Accepted November 22, 1995.
J12397

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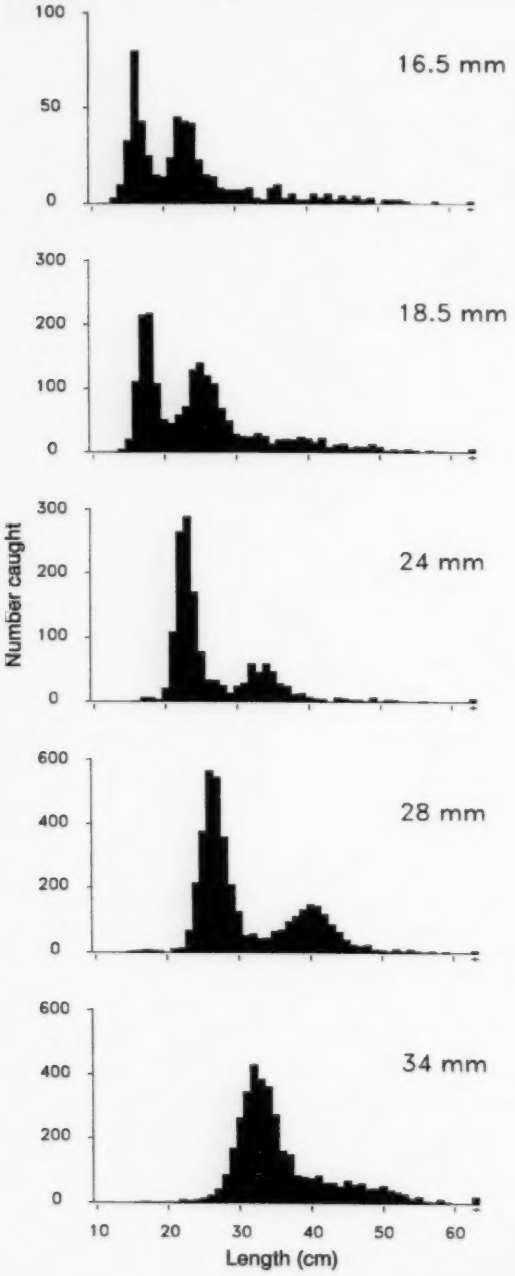
Fig. 1. Length–frequency distributions of cod catches in 1987–1993 for the five mesh sizes used. The plus sign indicates cod longer than 60 cm. In total, 11 602 cod were caught.



The nets used were made of grey monofilament nylon mounted with a hanging ratio of 0.5. In 1985–1986, three mesh sizes were used: 16.5, 24, and 33 mm bar length with twine diameters of 0.24, 0.38, and 0.33 mm, respectively. In 1987, two extra mesh sizes were added: 18.5 and 28 mm with twine diameters of 0.20 and 0.28 mm, respectively. Slight changes in the largest mesh size occurred over the survey period: in 1985 it was 33 mm; in 1986–1989 a mixture of 33 and 35 mm was used; and from 1990 to 1993 it was 34 mm. However, the twine diameter of the largest mesh size was kept constant at 0.33 mm. In all calculations the largest mesh size has been set to 34 mm. Except for some observations on how fish were caught in 1986 the analysis is confined to the 1987–1993 period in which all five meshes were used.

The nets were designed to match a mounted size of 8.0 m length and 1.6 m depth. The nets were rigged with float lines of a buoyancy of 13 g/m and lead lines of 50 g/m. The lengths of the float and lead lines were 10 m, thus leaving an open space of 2 m where the individual nets were tied together. This design was chosen to restrict the effect of larger fish being led by a small mesh-size net into a neighbouring net of a more suitable size. The five different mesh sizes were combined in random order and two such strings were then randomly chosen and tied together. Therefore, the experimental fleet of nets was composed of 10 nets with total dimensions of about 1.6×100 m.

Fishing stations were selected in shallow bays and inlets where the nets were set from an inflatable rubber boat equipped with a portable echo sounder. It was attempted to set the individual fleets of nets at constant depth and to spread effort evenly among depth strata of 0–5, 5–10, 10–15, and 15–20 m. Fishing was carried out both day and night with an average fishing time of 7.8 h. The total number of valid sets was 1250.

Catches

Catches were sorted by mesh size and measured to the nearest centimetre below. Fish that could not be associated with mesh sizes, i.e., fish falling out during the handling of the nets, were excluded from the analysis. However, these fish amounted to less than 3% of the catch. In total the analysis is based on 11 602 cod.

Figure 1 shows the length distribution of the catches by mesh size. It was observed that the majority of cod were captured either by being gilled or enmeshed by their maxillae. In three cases (the Nuuk area in 1986 and 1993 and the Qaqortoq area in 1987), all cod caught were individually examined for method of retention and classified into groups of gilled, maxillae caught, or otherwise entangled (Fig. 2). The bimodal distribution in the length distributions could clearly be attributed to how the cod were caught, with the primary mode being totally dominated by gilled cod whereas the secondary mode was composed of maxillae-caught cod. Cod otherwise entangled accounted for very few of the fish caught.

The 18.5-mm mesh size caught substantially more cod than the 16.5-mm mesh size (1899 and 539, respectively). The catches in these two meshes were composed of fish of about equal size; therefore, this difference is attributed to a difference in fishing power.

Estimation procedures

Estimation of selection characteristics

The relationship between the sizes caught and the mesh sizes is based on the principle of geometrical similarity (Baranov 1948). According to this axiom, gill-net selectivity depends only on the geometry of the fish relative to that of the mesh. This implies that the selection curves for different mesh sizes may be brought to the same form when fish size is normalized by mesh size. The best measure of size may be girth, which can be directly related to mesh perimeter. In the present investigation, length was used because it is far easier to measure. However, length and girth could be related by proportionality (maximal girth = 0.486(length), $R^2 = 0.94$, $n = 128$).

The bimodality in the catch distributions (Figs. 1 and 2) suggests that the length frequencies may be described by the sum of two normal density normal density distributions, ϕ_1 and ϕ_2, i.e.,

$$(1) \qquad C = A\phi_1(m_1, s_1) + B\phi_2(m_2, s_2)$$

where m_1, m_2, s_1, and s_2 are the means and standard deviations of two

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normal distributions and A and B are constants. By including a term of stochastic noise in eq. 1 the parameters associated with the two distributions may be estimated by nonlinear regression analysis. However, these parameters do not represent an unbiased selection curve as the catch frequencies depend on both the selection and the stock size distribution.

To estimate selection, knowledge or inference of fish abundance by size groups is required. Hamley (1975) expressed catch as a function of fish abundance (N), effort (E), and selection (S), as

$$(2) \quad C_{ms,l} = N_l E_{ms} S_{ms,l}$$

where l and ms refers to length and mesh size, respectively. When effort is the same for all mesh sizes, as is the case when using fleets of nets of different mesh sizes but of equal overall dimensions, effort will not influence the shape of the length distribution in the catches and hence may be ignored. The potential impact of differences in fishing power (P) between net sections has to be added to the model, i.e.,

$$(3) \quad C_{ms,l} = N_l P_{ms} S_{ms,l}$$

As the catch distribution suggested that the selection curve can be composed by summing two normal distributions the concrete model may be formulated as

$$(4) \quad C_{ms,l} = N_l P_{ms}(A\phi_1 + B\phi_2)$$

where $\phi_1 = \exp(-0.5(l - m_1)^2/(s_1)^2)$ and $\phi_2 = \exp(-0.5(l - m_2)^2/(s_2)^2)$. Equation 4 cannot be linearized but estimates may be obtained by nonlinear regression techniques. Rewriting eq. 4 by expanding it to cover different years and including stochastic noise ($\varepsilon_{y,ms,l}$) leads to

$$(5) \quad C^y_{ms,l} = N^y_l P_{ms} S_{ms,l} + \varepsilon_{y,ms,l}$$

The least squares estimate of this expression is

$$L = \sum_y \sum_{ms} \sum_l \left[C^y_{ms,l} - N^y_l P_{ms} S_{ms,l} \right]^2$$

to be minimized with respect to N^y_l, i.e.,

$$\frac{\partial L}{\partial N^y_l} = -2 \sum_{ms} \left[C^y_{ms,l} - N^y_l P_{ms} S_{ms,l} \right] S_{ms,l} P_{ms}$$

Solving for N^y_l gives

$$N^y_l = \frac{\sum_{ms} C^y_{ms,l} P_{ms} S_{ms,l}}{\sum_{ms} S^2_{ms,l} P^2_{ms}}$$

This estimate of the annual length distributions is regarded as a nuisance parameter and is introduced in eq. 5 for the subsequent estimations of the selectivity parameters and power parameters. Equation 5 may then be reduced to

$$(6) \quad C^y_{ms,l} = P_{ms} S_{ms,l} \cdot \frac{\sum_{ms} C_{ms,l} P_{ms} S_{ms,l}}{\sum P^2_{ms} S^2_{ms,l}} + \varepsilon_{y,ms,l}$$

from which the parameters can be obtained by least squares minimization.

The model requires 11 parameters to be estimated and 6 to describe selection (i.e., $([m_1,s_1],[m_2,s_2])$, which relate selection to fish size for the two capture processes; the parameters (A, B) that measure the efficiency of the two capture processes; and 5 parameters to account for the fishing power of the different nets. As the efficiencies and powers can only be expressed relative to each other the minimization was carried out with the constraints $P_{34} = 1$ and $A = 1$. As discussed below the use of the model resulted in estimates where the parameters were highly correlated, which implied that a number of different parameter sets provided almost equally good statistical fits between model and observations. Therefore, it was necessary to fix one parameter. All computations were carried out using the NLIN procedure in SAS (SAS Institute Inc. 1991).

Fig. 2. Length–frequency distributions of cod by mesh size and method of capture from the experiments in which the method of capture was recorded. In total, 1266 cod were caught in these experiments of which 941 were gilled, 316 were enmeshed by their maxillae, and 9 were otherwise entangled.

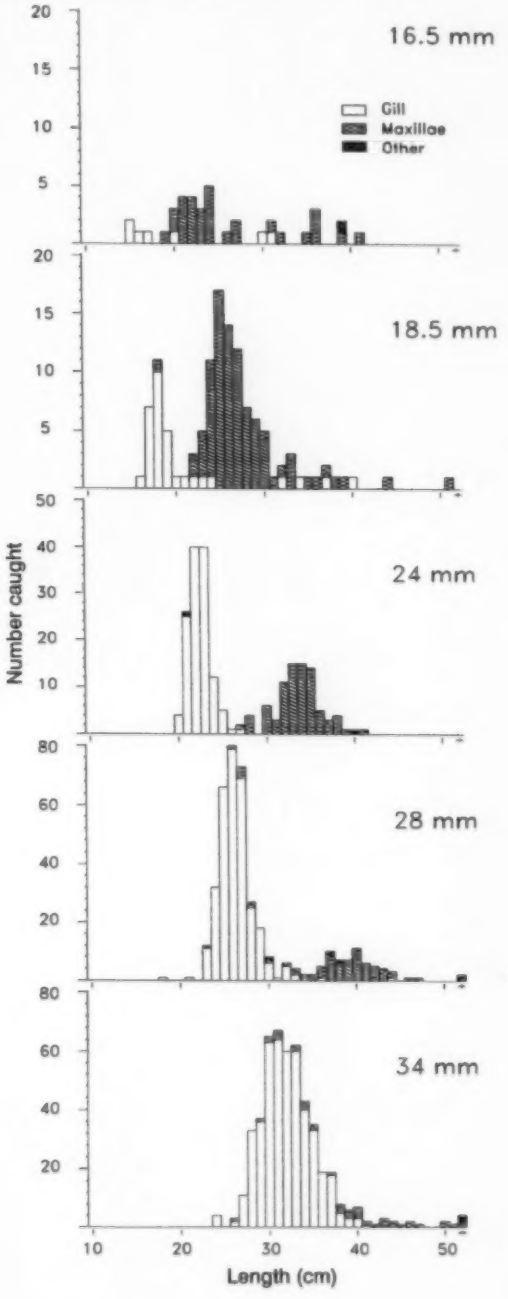

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Table 1. Mean length and standard deviation on the normalized scale (length / mesh size) estimated from the catch frequencies (Fig. 1) and from experiments where the retention method was recorded (Fig. 2).

			Normalized length (cm)	
Data source	Estimation method	Catch process	Mean	SD
All catches	Nonlinear regression	Gilled	0.949	0.065
		Maxillae enmeshed	1.362	0.181
Retention method noted	Direct	Gilled	0.947	0.111
		Maxillae enmeshed	1.398	0.248
	Nonlinear regression	Gilled	0.934	0.067
		Maxillae enmeshed	1.378	0.162

Table 2. Regression statistics, parameter estimates, and correlations between the estimated parameters derived by the nonlinear regression model given in eq. 6.

(A) Nonlinear least squares summary statistics.

Source	df	Sum of squares	Mean square
Regression	8	522 945	65 368
Residual	1637	14 318	8.75
Uncorrected total	1645	537 263	
(Corrected total)	1644	455 956	

(B) Parameter estimates.

			Asymptotic	Asymptotic 95% CI	
Effect	Parameter	Estimate	SE	Lower	Upper
Maxillae efficiency	B	0.157	0.007	0.141	0.173
Power 16 mm	P_{16}	0.397	0.036	0.327	0.467
Power 18 mm	P_{18}	1.274	0.096	1.085	1.462
Power 24 mm	P_{24}	0.440	0.015	0.410	0.469
Mean (gilled)	m_1	0.948	0.001	0.947	0.950
Mean (maxillae)	m_2	1.398	0.004	1.391	1.406
SD (gill)	s_1	0.066	0.001	0.065	0.067
SD (maxillae)	s_2	0.174	0.004	0.165	0.182

(C) Asymptotic correlation matrix.

	B	P_{16}	P_{18}	P_{24}	m_1	m_2	s_1	s_2
B	1	−0.67	−0.82	−0.33	0.30	0.16	0.32	0.03
P_{16}		1	0.88	0.57	−0.65	−0.32	−0.27	0.13
P_{18}			1	0.54	−0.68	−0.38	−0.21	0.25
P_{24}				1	−0.63	−0.36	0.15	0.03
m_1					1	0.23	0.15	−0.20
m_2						1	0.03	0.32
s_1							1	−0.30
s_2								1

Note: Maxillar efficiency is expressed relative to gilling set at a value of 1. The powers of the different mesh-size nets are expressed relative to the 28- and 34-mm mesh-size nets, which were assumed equal and set at 1.

Results

Identifying the modes to the catching process

The catches of the different mesh sizes (Fig. 1) were brought to the same scale by normalizing length with mesh size. Subsequently the parameters describing the two modes were estimated by eq. 1 (Table 1). For comparison, direct estimates of the parameters associated with gilling and maxillae enmeshing were obtained from the experiments where the way of retention was noted (cf. Fig. 2). This was done by normalizing length with mesh size and computing the means and standard deviations for gilled and maxillae-caught cod separately (Table 1). The two set of means were almost equal, but the standard deviations were about 50% higher in the direct estimations. If instead the parameters were estimated from the direct experiment but the information on how the fish were caught was ignored (i.e., using eq. 1), estimates similar to those determined from the entire data set were obtained (Table 1). This suggests that the decomposition of the length frequencies into normal distributions leads to the identification of the two peaks corresponding to the gilled and maxillae-caught cod but that the finer details of the catching method, represented by the outlying observations, may only be identified by actually observing how individual fish are caught.

Estimation of the selection curves and fishing power

The nonlinear regression fit of eq. 6 resulted in highly correlated parameter estimates (all estimated correlation coefficients were above 0.95). This indicated that the solution was not unique, i.e., that infinitely many equally good solutions existed. The reason for the lack of unique solutions is that the model basically estimates the product of selection and fishing power, and this means that any specific solution depends on the contrast found in the actual data.

Trials with the model showed that to derive unique solutions it was necessary to fix one parameter. The necessary supplementary constraint was based on Baranov's (1948) assertion that the fishing power of a gill net depends on the ratio of twine diameter to mesh size. By assuming this relationship one can fix the power of the 28-mm mesh as equal to the power of the 34-mm mesh, as the thread to mesh size ratios of these two meshes are about equal.

The solution found under this constraint explained approximately 97% of the total variability and at the same time it resulted in reasonable levels of parameter correlations (Table 2). The power of the 16.5- and 24-mm netting was estimated at less than half the value of the 28- and 34-mm meshes (which was set to 1.0). The efficiency of maxillae enmeshing, i.e., the amplitude of the maxillae capture process relative to gilling, was estimated at 15.7%. Inserting the estimated values of power and efficiency into the expression $S = P_{ms}(\phi_1 + B\phi_2)$ allows one to draw selection curves for individual meshes as well as the aggregated selection (Fig. 3). The aggregated selection in 1985–1986, when only three mesh sizes were in use, was evidently unsatisfactory because "blind holes" were found

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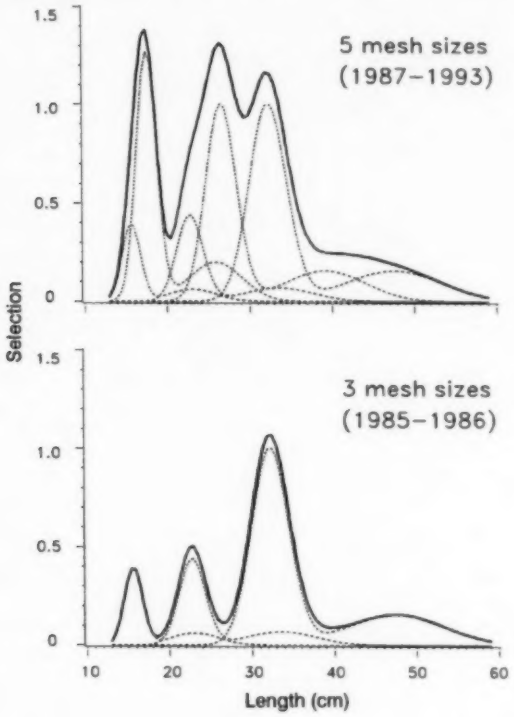
Fig. 3. Estimated selection curves for cod. The broken lines show the selection by individual mesh sizes whereas the solid line shows the aggregated selection.



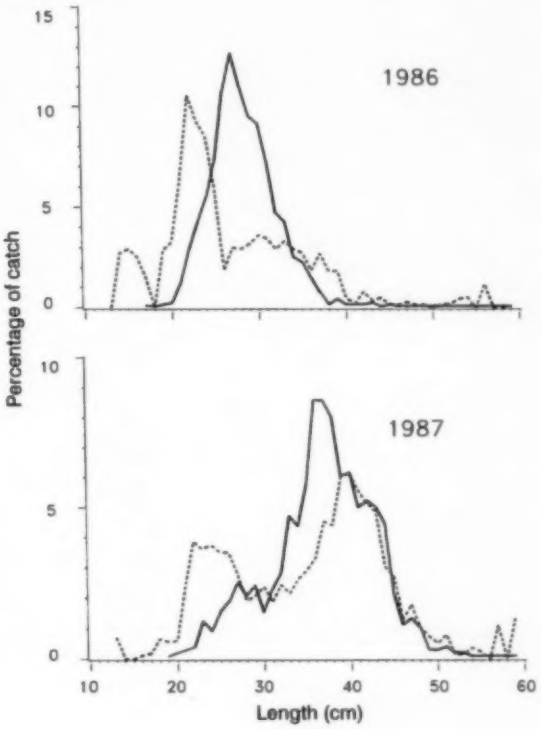
in between the mesh sizes for cod lengths of about 18 and 26 cm. This was recognized at the time and led to the introduction of the two extra mesh sizes in 1987 (Hovgård 1988).

Model validation

The estimated length frequencies in the stock (N_l) can be obtained from the total catch (C_l) and the aggregated selection (S_l), i.e., $N_l = C_l/S_l$. For 1986 and 1987 the length distribution estimated in this way can be compared with observed length distributions from hook and line jigging operations available for these years (Fig. 4). Jigging was conducted using small hooks (gape size 12–17 mm) and took place in the same area and at the same time as the gill-net experiments when time permitted. For 1986 the two length frequencies were in agreement in that the cod population was confined to fish between 20 and 40 cm in length, but the gill-net experiments predicted a modest abundance of cod around 25 cm where the hook catches peaked. However, as noted, the gill-net selection in 1986 was poorly determined for sizes around 25 cm; therefore, the abundance found at this size was estimated with low precision. The correspondence between the two length distributions was better for 1987, when the gill-net estimate was based on five mesh sizes.

The selectivity of the hooks is unknown; therefore, it is unclear how well the hook catches reflect the stock size distribution. However, the size distribution of the hook catches agrees with information from other sources. The peak of cod

Fig. 4. The length distribution estimated from the gill-net catches in 1986 and 1987 (broken lines) compared with the observed length distribution from concurrent hook catches (solid lines). The numbers caught with hooks were 1144 and 955 in 1986 and 1987, respectively.



between 20 and 40 cm in 1986 reflects the unusually strong year-class of 1984, and the lack of larger cod agrees with the fact that the year-classes of 1982 and 1983 were extremely weak (Buch et al. 1994). The hook catches in 1987 showed the 1984 year-class at age 3 years (30–50 cm) and the incoming year-class of 1985 (20–40 cm).

Discussion

The present work showed that the gill-net selection for cod could be explained by the aggregated effect of fish being gilled and caught by the maxillae. The identification of these capture processes was derived by observing how individual fish were caught (Fig. 2) but could also be clearly detected from the length distribution of catches (Fig. 1).

The mode of capture provides valuable information on how to interpret or model selection (McCombie and Berst 1969; Hamley and Regier 1973; Marais 1985). In most gill-net selection work, however, little consideration is given to how fish are actually caught and selection is typically assumed to follow some sort of a right-skewed distribution. In this respect it is worrying to note that when applying traditional graphical screening procedures to the present data the bimodality in selection is easily overlooked (Fig. 5).

Both gilling and maxillae enmeshing are well known for

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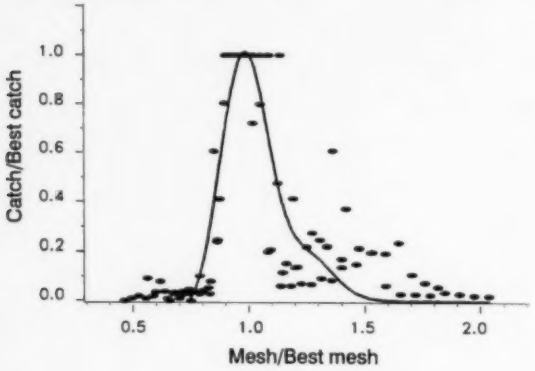
Fig. 5. A Gulland–Harding (1961) selection plot derived when information on the method of capture is ignored. The right-skewed selection curve was drawn by a truncated Gram–Charlier series as suggested by Regier and Robson (1966).



many species (Hamley 1975), although the nomenclature used to describe maxillae catching is somewhat confusing (fish being entangled, tangled, snagged, meshed, caught, attached, wedged, or held by their maxillae, premaxillae, jaw, snout, or lips). In a few cases the importance of this capture process is quantified (e.g., Marais 1985), but most often the information given may at best be the number gilled compared with those otherwise caught (e.g., Olsen and Tjemsland 1963; Helser et al. 1991). For cod, previous gill-net studies indicate that maxillae enmeshing is of little importance (Rollefsen 1953; Hylen and Jacobsen 1974; Clay 1981). These studies, however, were all based on large, commercially used mesh sizes (83–93 mm bar length), whereas the mesh sizes used in the Greenland survey were chosen to target prerecruit cod only. As larger fish disappear through mortality, one can expect that the modes of a large mesh size will be much less distinctive than those of a small one. This phenomenon will especially affect maxillae enmeshing, which requires the availability of cod that are about 1.4 times the size of those being gilled by the same mesh size. Therefore, for a small experimental mesh size, one may end up observing a distinct bimodal length distribution, whereas a large commercial mesh size may only produce a single mode of gilled fish and some scattered catches of very large individuals. In a number of other studies, bimodal length distributions are indicated for smaller mesh sizes whereas only one mode may be detected for the larger meshes (Gulland and Harding 1961; Kawamura 1972; Erhard and Die 1988). Similarly, Winters and Wheelers (1990) noted that herring caught by the snout were only of importance in the smallest mesh sizes used. This suggests that if one wishes to identify different capture processes, it may be prudent to use small meshes targeting sizes of fish still abundant in the sea.

Both the gilling and the maxillae-capture processes could be described by normal distributions with means and standard deviations proportional to mesh size. This formulation is conceptually simple and in strict accordance with the principle of geometrical similarity. Helser et al. (1991) restricted modelling to gilled fish only, and fitted mean, standard deviation, and a skewness parameter to individual mesh sizes. They concluded that the selection curves were not of the same form for all mesh sizes. However, the limited difference found in the shape of the selection curves between those mesh sizes that caught an acceptable number of fish suggests that the gilling capture process may probably be equally well described by the use of nonskewed normal distribution with mean and standard deviation being proportional to mesh size.

The estimating procedure uses the fact that the size distribution of the catches is the product of size distribution in the stock and selection. This procedure is conceptually similar to the classical estimation methods of McCombie and Fry (1960) and Kitahara (1970) where selection is estimated by graphically adjusting catches from individual length groups to fit a common master selection curve. The array of adjustment factors applied to the different length groups in these methods is in fact an implicit expression of the size distribution of the stock.

The estimated relationship between mesh size and sizes of fish captured indicates a high degree of selection for both capture processes. The use of the normal distributions in the model formulation allows simple interpretations of the degree of selection, e.g., by measuring selection range by the ratio of the standard deviation to the mean. For gilling and maxillae enmeshing these ratios were 0.07 and 0.12, respectively. This implies that about 70% of the gilled cod were of a size deviating less than 7% from the modal length.

The estimations further showed that the efficiency parameter of maxillae capture (B) was only 0.16 times that of gilling. However, B measures only the maximal selectivity without considering the difference in selection range associated with the two capture processes. Including the effects of differences in selection ranges by comparing A times the standard deviation for gilling with B times the standard deviation for maxillae capture, i.e., by comparing the areas under the two selection curves, implies that the maxillae-capture process is about 0.41 times as efficient as gilling.

The selection model differs from other models in that it includes parameters of fishing powers of individual mesh sizes in addition to the selection parameters. In practice, however, it was necessary to add the constraint that the powers of the 28- and 34-mm mesh sizes were equal assuming that the powers were equal when the ratios between twine diameter and mesh size were the same (Baranov 1948). The fishing powers of the 16.5- and the 24-mm mesh sizes were estimated at less than half the 28- and 34-mm value, whereas the power of the 18.5-mm net was estimated as slightly above it (Table 2). These results indicate that the fishing power increases when the ratio between twine diameter and mesh size decreases. This is in accordance with the few direct studies on this matter that suggest that thin twines fish better than thicker ones (Predel 1963; Steinberg 1985; Hansen 1974 (for peamouth (*Mylocheilus caurinus*) but not for perch (*Perca flavescens*))). Use of the present method to indirectly estimate power differences between nets for other species taken in the Greenland gill-net survey also produces similar estimates to those found for cod (Hovgård 1996).

Acknowledgments

The author is very grateful to Hans Lassen for valuable support in the development of the nonlinear regression model and for constructive criticism during the preparation of the manu-

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script. Warm thanks are also given to the former skipper of R/V *Misiliisoq*, Aage Pedersen, for his help in the development of the gill-net survey.

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Effect of twine diameter on fishing power of experimental gill nets used in Greenland waters

Holger Hovgård

Abstract: The relative fishing powers of experimental gill nets were estimated for shorthorn sculpin (*Myoxocephalus scorpius*), Greenland cod (*Gadus ogac*), and Atlantic cod (*Gadus morhua*). The results suggested that fishing power was negatively correlated to the ratio between twine diameter and mesh size. Similar differences in fishing power could be inferred for American plaice (*Hippoglossoides platessoides*) where no mesh-size selection was evident.

Résumé : Le pouvoir pêchant relatif de filets maillants expérimentaux a été évaluée dans le cas du chaboisseau à épines courtes (*Myoxocephalus scorpius*), de l'ogac (*Gadus ogac*) et de la morue franche (*Gadus morhua*). D'après les résultats, il existerait une corrélation négative entre le pouvoir pêchant et le rapport entre le diamètre du fil et la taille de la maille. Des différences similaires au niveau du pouvoir pêchant pourraient être déduites dans le cas de la plie canadienne (*Hippoglossoides platessoides*) lorsqu'aucune sélection de la taille de la maille n'est évidente.
[Traduit par la Rédaction]

Introduction

Gill nets are commonly used in scientific surveys to estimate the size distribution of fish populations. However, gill nets are highly size selective, with most fish caught often deviating less than 20% from the optimal fish length. To cover the full size range of a population it is necessary to use a fleet of nets with different mesh sizes, and a number of scientific studies have been concerned with how to design optimal fleets for experimental fisheries (e.g., Reiger and Robson 1966; Jensen 1986). The fishing power of the gill nets in the fleet must be standardized by using the same net material and the same gear rigging. There are, however, uncertainties as to how to standardize twine thickness because of insufficient knowledge about how this factor affects selectivity (Hamley 1975).

Annual gill-net surveys to estimate Atlantic cod (*Gadus morhua*) recruitment in Greenland waters suggested that there is a considerable difference in fishing power between nets made with different twine thicknesses (Hovgård 1996). In the present paper this analysis is extended to other species found in the Greenland survey.

Materials and methods

This study is based on data from annual gill-net surveys carried out in the coastal areas of southwestern Greenland from 1987 to 1993. The experimental gill nets were made of grey monofilament nylon with mesh sizes (bar length) of 16.5, 18.5, 24, 28, and 34 mm and having twine diameters of 0.24, 0.20, 0.38, 0.28, and 0.33 mm, respectively. For a more detailed description of the survey gear and the operational procedure, see Hovgård (1996).

The catches were dominated by Atlantic cod, shorthorn sculpin (*Myoxocephalus scorpius*), Greenland cod (*Gadus ogac*), and American plaice (*Hippoglossoides platessoides*); the total numbers caught were 11 602, 6578, 2550, and 1035, respectively. These totals do not include fish that could not be associated with mesh size (fish falling out after the nets were taken in), which accounted for less than 3% of the number caught except for American plaice where they amounted to 8.5%.

Size selection and fishing power were estimated by a two-step approach developed on the cod data (Hovgård 1996). In the initial step a selection pattern was identified from the length frequencies of catches taken in the different mesh sizes. This selection pattern was subsequently used in the second step where the parameters of selection and fishing power were estimated by a nonlinear minimization routine. As the fishing powers can only be expressed relative to each other the minimizations were carried out by fixing the power of the 34-mm mesh at a value of 1.

As in the case of Atlantic cod it was found that the parameters were highly correlated, implying that different parameter sets provided about equally good fits between the model and the observations. To derive unique solutions it was necessary to fix one further parameter. On the basis of an assumption by Baranov (1948) this was done by giving equal fishing power to two mesh sizes (28 and 34 mm) having a roughly equal ratio between twine diameter and mesh size. This corresponds to assigning an equal height to the selection curves for these two meshes.

Results and discussion

The catches of Greenland cod showed clear bimodal length distributions (Fig. 1*a*). As in the case of Atlantic cod, this was caused by the individual fishes being either gilled or enmeshed at their maxillae. For the sculpin a bimodal catch distribution could only be seen for the two smallest meshes (Fig. 1*b*). The lack of a secondary peak for the larger mesh sizes may result from the lack of individuals of a suitable size in the population. For this species it was not possible to assess how individual fish were captured as they were most often held in the nets by their numerous spines. In contrast, no difference in the size distribution between mesh sizes could be detected for American plaice (Fig. 1*c*). However, the numbers caught in the different mesh sizes differed considerably with 120, 263, 87, 206, and 359 being taken in the 16.5-, 18.5-, 24-, 28-, and 34-mm meshes, respectively.

For Greenland cod and sculpin the catch distributions suggested that the selection curves may represent the sum of two

Received July 7, 1995. Accepted November 22, 1995.
J12985

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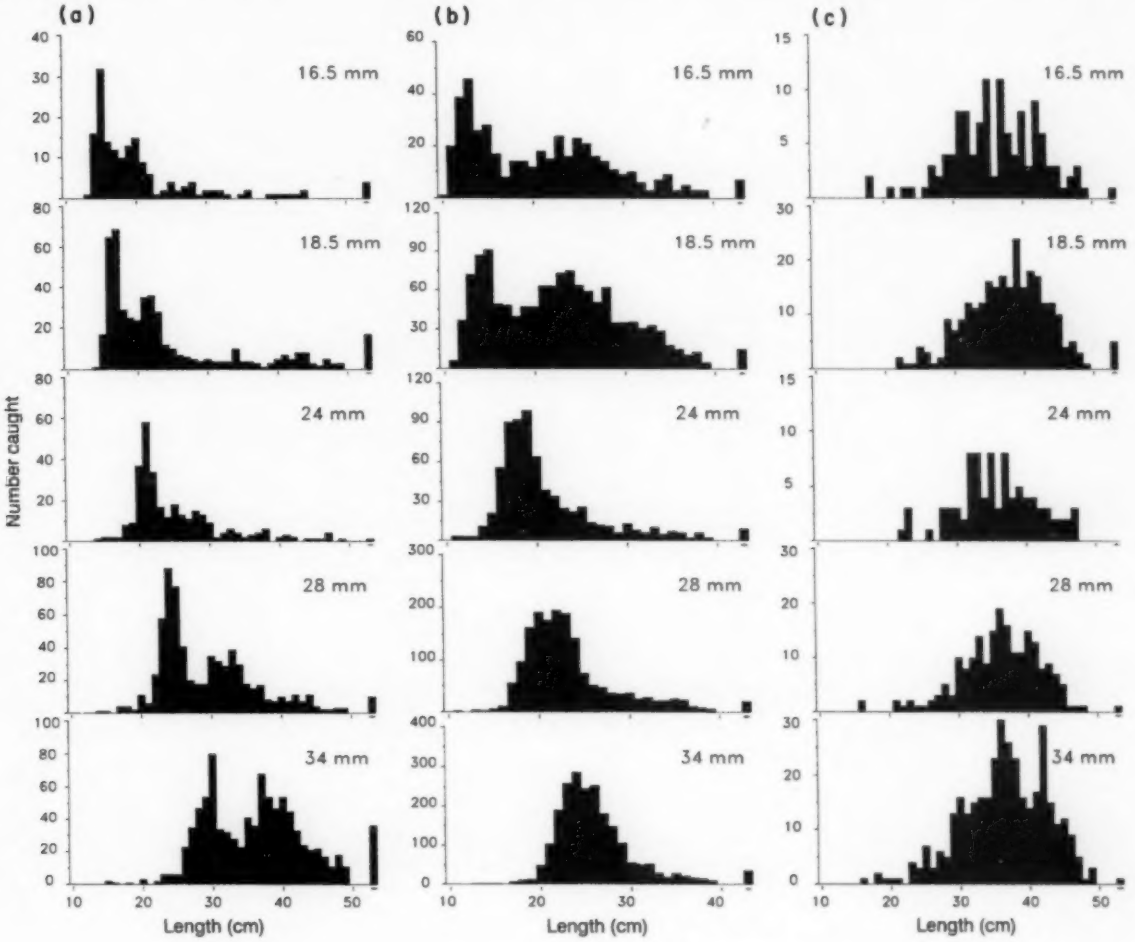
Fig. 1. Length–frequency distributions for (*a*) Greenland cod, (*b*) shorthorn sculpin, and (*c*) American plaice taken in the Greenland gill-net surveys, 1987–1993.



normal density distributions (ϕ_1, ϕ_2). When the fishing power of different mesh-size nettings (P_{ms}) is included, selection (S_{ms}) takes the form $S_{ms} = P_{ms}(A\phi_1 + B\phi_2)$, where A and B are constants.

The estimations, carried out by nonlinear regression analysis, showed that the relative efficiencies of the secondary catch process (parameter B relative to A) were about 0.16 and 0.34 for sculpin and Greenland cod, respectively (Tables 1 and 2). The sizes of Greenland cod taken at both peaks were considerably smaller than found for Atlantic cod (Hovgård 1996). This was caused by the greater plumpness of Greenland cod, and if selection was related to the ratio of girth to mesh perimeter instead of the ratio of length to mesh size, the size of maximal gilling efficiency was in fact about equal (1.16 for Atlantic cod, 1.19 for Greenland cod). The standard deviations of the normal distributions associated with both the primary and secondary capture modes were about three times higher for sculpin than for the two gadoids. This makes sense if one considers the many spines that cause entangling for the sculpin. For both sculpin and Greenland cod, the fishing power of the 18.5-mm net was considerable higher than that of the 16.5- and 24-mm mesh-size netting.

With no selection evident for American plaice a difference in fishing power may be assumed to be reflected directly by the numbers caught in the different nets. However, the total lack of any signs of selection in the catches may require some consideration. Information on gill-net selection for American place is not available, but if data for other flatfishes are applied, the maximal selection should be found at a length of about six or seven times the bar length (Olsen and Tjemsland 1963; Boje and Hovgård 1995; Holst and Moth-Poulsen 1995). With the mesh sizes used in the present experiment, this implies that maximal selection should be found at fish sizes of 10–24 cm. However, less than 3% of the American plaice were below 24 cm in length. This suggests that the lack of selection is due to a gross mismatch between mesh sizes and the sizes of fish in the area, i.e., that catches were just composed of randomly entangled large fish. The fact that large American plaice were

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Table 1. Regression statistics and parameter estimates for shorthorn sculpin, derived by the nonlinear regression model given in Hovgård (1996).

(A) Regression.

Source	df	Sum of squares	Mean square
Regression	8	124 379	15 546
Residual	1077	4 701	4.37
Uncorrected total	1085	129 081	
(Corrected total)	1084	92 906	

(B) Parameter estimates.

			Asymptotic 95% CI	
Effect	Parameter	Estimate	Lower	Upper
Primary mean	m_1	0.728	0.726	0.730
Secondary mean	m_2	1.279	1.221	1.337
Primary SD	s_1	0.089	0.086	0.091
Secondary SD	s_2	0.461	0.397	0.524
Secondary peak	B	0.159	0.138	0.180
Power 16 mm	P_{16}	0.730	0.605	0.856
Power 18 mm	P_{18}	1.547	1.351	1.744
Power 24 mm	P_{24}	0.590	0.550	0.629

Note: The efficiency of the secondary peak is expressed relative to the primary peak set at a value of 1.0. The fishing powers of the different mesh sizes are expressed relative to the values of the 28- and 34-mm meshes, which were assumed equal and set at 1.0.

Table 2. Regression statistics and parameter estimates for Greenland cod, derived by the nonlinear regression model given in Hovgård (1996).

(A) Regression.

Source	df	Sum of squares	Mean square
Regression	8	18 917	2365
Residual	1357	1 510	1.11
Uncorrected total	1365	20 427	
(Corrected total)	1364	15 918	

(B) Parameter estimates.

			Asymptotic 95% CI	
Effect	Parameter	Estimate	Lower	Upper
Primary mean	m_1	0.861	0.859	0.864
Secondary mean	m_2	1.101	1.086	1.116
Primary SD	s_1	0.044	0.041	0.048
Secondary SD	s_2	0.157	0.145	0.169
Secondary peak	B	0.342	0.302	0.382
Power 16 mm	P_{16}	0.673	0.510	0.837
Power 18 mm	P_{18}	1.082	0.881	1.282
Power 24 mm	P_{24}	0.547	0.484	0.610

Note: The efficiency of the secondary peak is expressed relative to the primary peak set at a value of 1.0. The fishing powers of the different mesh sizes are expressed relative to the values of the 28- and 34-mm meshes, which were assumed equal and set at 1.0.

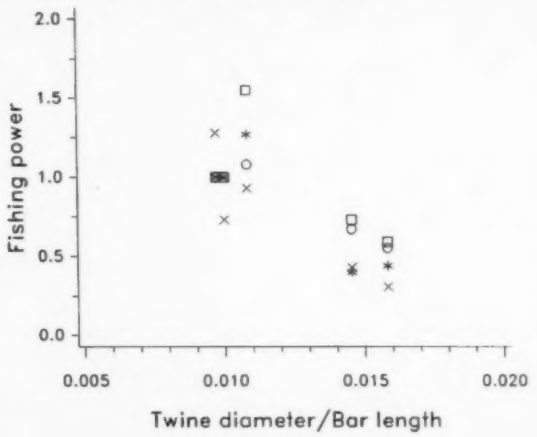
found in the area matches the fact that spawning takes place in shallow inshore waters during May and June (Smidt 1979).

The available information, including the inference of the numbers of American plaice made directly from the catches, indicates that the fishing power of the various nettings is negatively correlated with the ratio between twine diameter and mesh size (Fig. 2).

In general it is assumed that the fishing power depends on factors affecting the physical attributes of the nets such as elasticity, strength, colour, and visibility. A number of studies on the effect of twine material have been carried out in conjunction with the replacement of cotton yarn with nylon yarn and later with monofilament nets (see Hamley 1975 for a review). However, the effect of twine thickness, which is the only factor varying between mesh sizes in the present experiment, is insufficiently understood. Baranov (1948) assumed that two meshes were equally efficient when the ratio between twine diameter and mesh size was the same but provided no empirical evidence; it is not it clear whether Baranov assumed that fishing power was inversely proportional to this ratio.

Empirical studies of the importance of twine diameter are few. Predel (1963) compared two 24-mm mesh-size nets with twine diameters of 0.23 and 0.38 mm and found that the finer twine caught between 49 and 57% more *Coregonus albula* than did the thicker twine. Hansen (1974), who used 38-mm stretched mesh and two twine diameters (0.133 and 0.267 mm), found the highest catches of peamouth (*Mylocheilus caurinus*) for the thin twine (81% more than for the thick twine) whereas the opposite was seen for yellow perch (*Perca flavescens*) (thin twine caught 11% less than the thick twine). More importantly, Hansen found that the modal sizes were clearly different between twine diameters; fine twine caught larger fish than thick twine (the fish were 24 and 14% longer for peamoth and perch, respectively). Jensen (1995) described experiments in which nets with a twine diameter of 0.10 mm caught 2.2 times more Arctic char (*Salvelinus alpinus*) than nets made of 0.17-mm twine. Similarly, for multimonofilament trammel nets, a reduction in the number of threads from four to three resulted in an increase in Atlantic cod catches of

Fig. 2. Estimated fishing power plotted against the ratio of twine diameter to bar length for cod (asterisks), shorthorn sculpin (circles), Greenland cod (squares), and American plaice (crosses). The values for cod, sculpin, and Greenland cod are expressed relative to values for the 28- and 34-mm nets, which were assumed equal and set at 1.0. For American plaice, where no selection could be inferred, the power is derived as the number caught divided by the mean catch taken in the 28- and 34-mm mesh sizes.



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about 35% (Steinberg 1985). The present estimates agree with Baranov's (1948) assumption and the results of Predel, Steinberg, and Jensen. The findings of Hansen, suggesting a direct effect of twine thickness on selection, were not evident for any of the species in the present study.

In his review article on gill-net selection, Hamley (1975) stressed that when Baranov (1948) assumed that selection curves from different mesh sizes were of the same height, this required that the twine thickness was proportional to mesh size. Hamley further noted that many researchers have accepted Baranov's assumption without adjusting twine thickness and that experiments were needed to quantify the importance of twine diameter. Unfortunately, much recent research on gill-net selection lacks information on the twine thickness used.

Failing to incorporate the effect of fishing power in selection models may lead to various misinterpretations of experimental results. When indirect estimations of gill-net selection are made, i.e., when selection is inferred from comparisons of catches of the same length group taken by different mesh sizes, the estimated selection will inevitably be confounded by differences in fishing power. When applying direct estimation methods, i.e., when selection is estimated from the catches taken from a known (typically tagged) population, estimated behavioral aspects guiding encounter probabilities of different fish sizes may similarly be compromised. Therefore, it is suggested that more effort be given to estimating the powers of different nets. This may be done by applying procedures similar to those used in the present work or by directly comparing the effects of various twine thicknesses in field experiments.

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Experimental transmission of epidermal lip papillomas in white sucker, *Catostomus commersoni*

Peter D. Premdas and Chris D. Metcalfe

Abstract: A viral etiology is proposed for the development of epidermal papillomas in white sucker, *Catostomus commersoni*. Suckers were collected from the Ganaraska River at Port Hope, Ontario, where papilloma prevalence exceeds 46% and from the Squaw River at Buckhorn, Ontario, where papilloma prevalence is less than 5%. Tumour transmission was attempted by (*i*) holding nonpapillomatous Squaw suckers in the same tank as papillomatous Ganaraska suckers and by (*ii*) injecting Ganaraska papilloma filtrates into nonpapillomatous Squaw suckers. In the first study, approximately 20% of Squaw suckers developed small areas of hyperplasia on the lip epithelium. However, in the injection study, approximately 50% of surviving Squaw suckers developed true papillomas near the site of injection while less than 3% of control Squaw suckers developed similar tumours. Injections of organic contaminants extracted from Lake Ontario rainbow trout (*Oncorhynchus mykiss*) were unsuccessful in inducing papillomas or increasing the growth of existing papillomas. Induced papillomas were grossly and histologically similar to donor papillomas and exhibited altered protein kinase C and ornithine decarboxylase activity similar to that in the donor papillomas. No viruses were visible in either donor or induced papillomas under transmission electron microscopy.

Résumé : Les auteurs avancent l'hypothèse d'une étiologie virale pour expliquer la formation de papillomes épidermiques chez le meunier noir, *Catostomus commersoni*. À des fins de comparaison des taux de papillomes, ils ont capturé des meuniers dans la Ganaraska, à Port Hope (Ontario), et dans la Squaw, à Buckhorn (Ontario). Ils ont observé un taux supérieur à 46% chez les premiers, contre moins de 5% chez les seconds. Les auteurs ont tenté d'induire expérimentalement la transmission de tumeurs en (*i*) introduisant des meuniers exempts de tumeurs de la Squaw dans un bassin contenant des meuniers de la Ganaraska porteurs de papillomes et (*ii*) injectant des filtrats de papillomes de meuniers de la Ganaraska à des meuniers sains de la Squaw. Dans la première expérience, environ 20% des meuniers de la Squaw ont développé de petites lésions hyperplasiques au niveau de l'épithélium des lèvres. Dans la deuxième expérience, environ 50% des meuniers survivants de la Squaw ont développé un papillome véritable près du point d'injection, comparativement à seulement 3% des meuniers témoins de la Squaw. L'injection de contaminants organiques extraits de truites arc-en-ciel (*Oncorhynchus mykiss*) provenant du lac Ontario n'a pas induit la formation de papillomes ni accéléré la croissance des papillomes existants. Les papillomes dont le développement avait été induit expérimentalement étaient de manière générale et au plan histologique semblables aux papillomes des « donneurs » et présentaient, à l'instar de ces derniers, une altération de l'activité de la protéin kinase C et de l'ornithine décarboxylase. À la microscopie électronique par transmission, aucun virus n'a été observé, ni dans les papillomes des donneurs, ni dans ceux dont le développement avait été induit expérimentalement.
[Traduit par la Rédaction]

Introduction

Epizootics of external neoplasms, such as skin and lip papillomas, have been observed in fish from the Great Lakes (Harshbarger and Clark 1990). Although there is some evidence that high prevalences of external tumours in benthic fish are linked to exposure to chemical contaminants (Sonstegard 1977; Black 1983; Hayes et al. 1990; Bowser et al. 1991; Premdas et al. 1995), external tumours are also found on fish from relatively uncontaminated regions (Roberts and Bullock 1979; Smith and Zajlik 1987). Lip and body papillomas are the most common forms of neoplasia observed in white sucker, *Catostomus commersoni*, from the Great Lakes basin (Smith and Zajlik 1987; Smith et al. 1989*a*, 1989*b*). Lip papilloma prevalence ranges from 40 to 50% in suckers from contaminated regions of the Great Lakes, as compared with <5–16% in suckers from nonpolluted regions (Smith et al. 1989*a*, 1989*b*; Premdas et al. 1995).

A number of biotic and abiotic factors may influence the development of external neoplasms in fish and other lower vertebrates. Proliferation and regression of epidermal papillomas in the European eel, *Anguilla anguilla*, is dependent on both salinity and temperature (Peters and Peters 1977, 1979). Papillomatosis in Atlantic salmon, *Salmo salar*, and rainbow trout, *Oncorhynchus mykiss*, varies seasonally (Carlise 1977; Roberts and Bullock 1979). Seasonal temperature and geographic location also affect the prevalence and growth of epidermal papillomas in the Japanese newt, *Cynops pyrrhogaster* (Asahima et al. 1982, 1985). Crowding of white suckers held in the laboratory results in either growth of existing papillomas or development of new papillomas (Premdas and Metcalfe 1994).

Received March 28, 1995. Accepted November 20, 1995.
J12841

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Tumour-causing viruses may be a factor in the etiology of external tumours in fish. Herpesvirus capsids and C-type particles were found in epidermal hyperplasias affecting northern pike, *Esox lucius* (Yamamoto et al. 1985), and adenoviruslike particles were associated with epithelial hyperplasias affecting dab, *Limanda limanda* (Bloch et al. 1986). A C-type virus was implicated in the etiology of epidermal papillomas in white sucker (Sonstegard 1977), although this was not confirmed in later studies (Smith et al. 1989*a*, 1989*b*). A viral etiology is also suspected in papillomatosis affecting Atlantic salmon (Carlise 1977) and rainbow trout (Roberts and Bullock 1979), and in stomatopapillomas affecting the European eel, where several different viruses have been identified (McAllister et al. 1977; Pilcher and Fryer 1980*a*, 1980*b*).

External neoplasms in fish, such as lip papillomas or dermal lesions, may provide readily identifiable biomarkers for ecosystem health (Malins et al. 1987; Murchelano 1990; Baumann 1992). The white sucker has been proposed as a sentinel species for assessing ecosystem health in the Great Lakes (Munkittrick and Dixon 1989). It has been suggested that papilloma in white sucker may develop in response to viral infection (Smith et al. 1989*a*), but the prevalence and severity of this disease may be potentiated by chemical pollutants (Black 1983, 1984; Premdas et al. 1995). Sonstegard (1977) observed C-type viruses with a diameter of approximately 100 nm budding from the cytoplasmic membranes of cells taken from white sucker papillomas and detected reverse transcriptase activity in papilloma homogenates, implicating a retroviral etiology for these neoplasms. However, these results have not been validated.

This paper presents the results of a study designed to determine whether viruses are the primary etiological factors responsible for the development of papilloma in white sucker. Transmission of papilloma was attempted using two protocols: (*i*) horizontal transmission and (*ii*) injection of cell-free filtrates from papillomatous suckers. To determine whether contaminants found in Great Lakes fish influence the expression of papilloma, suckers were also injected with a complex mixture of chemical contaminants in an extract prepared from the muscle of Lake Ontario rainbow trout. These treatments were designed to determine whether organic contaminants are capable of inducing new papilloma or affect the development of existing papilloma.

Materials and methods

Sampling

White sucker were captured during the annual migratory spawning run in spring (mid- to late April) of 1992 and 1993. Fish were collected from two populations in central Ontario that migrate up the Ganaraska River at Port Hope and the Squaw River at Buckhorn. The Ganaraska River discharges into Lake Ontario, which is contaminated with a variety of toxic chemicals (Baumann and Whittle 1988). The Squaw River, situated in rural central Ontario, discharges into Little Bald Lake, which is part of a chain of small lakes generally classified as pristine.

Water temperatures fluctuated between 10 and 12°C during sampling, because of heavy seasonal rains. Dipnetting and electrofishing were the primary means of capture employed at these sites. Animals were aged, weighed, and measured as described previously (Premdas and Metcalfe 1994; Premdas et al. 1995). Ganaraska and Squaw suckers either bearing or not bearing lip papillomas were transported to Trent University, Peterborough, Ont., and held in fiberglass tanks receiving Otonabee River water (Peterborough, Ont.) maintained at 10–12°C. An 8 h dark : 12 h light photoperiod was used for the duration of the experiments.

Papilloma transmission and induction studies

Tumour transmission was attempted using two protocols: (*i*) by placing nonpapillomatous suckers in the same tank as papillomatous suckers (horizontal transmission study) and (*ii*) by subdermal injection of cell-free filtrates from sucker papilloma into suckers not initially bearing papilloma (cell-free filtrate study).

In the horizontal transmission study, which was conducted in 1992, random samples of 30 papillomatous suckers from the Ganaraska River were placed together in a single tank with 20 nonpapillomatous Squaw River suckers. Fish density was estimated at approximately 60 fish/m^3. Control groups of 25 nonpapillomatous Ganaraska River suckers (control A) and 20 nonpapillomatous Squaw River suckers (control B) were placed in separate tanks at densities of 30 and 23 fish/m^3, respectively, under laboratory conditions similar to those described previously (Premdas and Metcalfe 1994; Premdas et al. 1995). The mean age, length, and weight were 6 years, 40.6 cm, and 1.15 kg for Ganaraska River suckers and 5 years, 33.7 cm, and 1.06 kg for Squaw suckers. Suckers were lightly anaesthetized with MS–222 (3-aminobenzoic acid ethyl ester) and tagged near the dorsal fin area with spaghetti tags that were numbered and colour coded. The numbers, size, and location of all lip papillomas were noted. As a prophylactic against latent bacterial or fungal infection, Borgal™ antibiotic (40 mg trimethoprim, 200 mg sulfadoxine/mL), sold by Hoechst Canada Inc., was injected into the muscle adjacent to the dorsal fin (50 µL/kg body weight). Fish were then acclimated and starved for 10 days before being fed ad libidum every 3 days with No. 5 size trout chow (Purina). Antibiotic treatments and papilloma measurements were carried out every 14 days.

At the end of these experiments, suckers were killed by an overdose of MS–222 and examined. After data on the size (diameter and height) and location of lip papillomas were recorded, papilloma and normal epidermal tissue were removed from the Ganaraska and Squaw suckers and either placed in tissue cassettes and transferred to Bouin's fixative for histological analysis or placed into 3% buffered gluteraldehyde (pH 7.2) for electron microscopy. No tissues from these experiments were prepared for biochemical analysis.

In the cell-free filtrate study conducted in 1993, random samples of 121 Squaw River suckers that were not initially bearing papilloma and 20 papillomatous Ganaraska River suckers were transported back to Trent University. The average age, length, and weight of fish were 5 years, 33.7 cm, and 1.4 kg for Squaw suckers and 7 years, 43.4 cm, and 1.06 kg for Ganaraska suckers. Squaw and Ganaraska suckers were placed in separate fiberglass or polyethylene tanks at fish densities of approximately 12–30 and 11–30 fish/m^3, respectively, and acclimated for 10 days before being fed initially. Upon arrival, animals were lightly anaesthetized with MS–222, tagged near the dorsal fin area, and treated with Borgal™ antibiotic as described previously.

Captive Ganaraska River suckers were examined weekly and any lip papillomas were measured and their locations recorded. Proliferating papillomas, defined as papillomas that had increased in size since the last measurement, were removed and frozen in liquid nitrogen or stored on ice (15 min maximum) and used for preparing cell-free filtrates of papilloma tissue. Cell-free filtrates were also prepared from papillomas removed on site from spawning Ganaraska River suckers and immediately frozen in liquid nitrogen. Filtrates were also prepared from dorsal muscle tissue of hatchery-raised juvenile rainbow trout as a control treatment. To prepare cell-free filtrates, frozen or fresh tissue was weighed and immediately homogenized on ice in an equal volume of homogenizing medium consisting of four parts ice-cold phosphate-buffered saline (PBS) and one part dimethyl sulfoxide (DMSO). The homogenate was then centrifuged at 15 000 rpm for 7 min at 4°C. The supernatant was decanted and pressure filtered

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through a cold sterile Millex®-GS 0.22-μm filter unit (Millipore, Bedford, Mass.). The cell-free filtrate was immediately placed on ice and used within 1 h.

When possible, papilloma from one donor (Ganaraska) fish was used to prepare cell-free filtrate to inject all suckers in a single experimental group. When papilloma from one fish was not large enough to produce sufficient quantities of filtrate, smaller batches of filtrate were individually prepared from several fish. However, filtrate batches were not pooled. When the filtrate was injected into an experimental group, each batch was used to inject as many suckers as possible so that each fish received filtrate from only one (but not necessarily the same) donor fish. Filtered ice-cold homogenizing medium was used in all control injections. Squaw suckers not initially bearing papilloma were lightly anaesthetized with MS–222 and injected subdermally into the upper lip with 20 μL of cell-free filtrate prepared from papilloma of wild Ganaraska River suckers (25 fish) or proliferating papilloma from captive Ganaraska suckers (50 fish). A total of 17 fish were injected with 20 μL of trout muscle cell-free filtrate to determine if papilloma could be induced by an immunological reaction. Controls (35 fish) received 20 μL of filtered ice-cold homogenizing medium. Injections were repeated every 14 days for 10 weeks (five injections).

At the end of these experiments, Squaw suckers were killed by an overdose of MS–222. Any papillomas were measured, and locations on the lip recorded. Normal epidermal tissue and any papillomas that were present were removed and either placed in tissue cassettes and transferred to Bouin's fixative for histological analysis, placed in 3% buffered gluteraldehyde (pH 7.2) for electron microscopy, or frozen in liquid nitrogen for biochemical analysis.

In the contaminant injection study, which was conducted in 1993, a random sample of 20 papillomatous and 20 nonpapillomatous Ganaraska suckers were placed in separate tanks at densities of approximately 11–30 fish/m^3, and acclimated for 10 days before being fed initially. The mean age, length, and weight of these suckers were 7 years, 43.3 cm, and 1.68 kg. Upon arrival, animals were lightly anaesthetized with MS–222, tagged near the dorsal fin area, and treated with Borgal antibiotic as previously described. In this study, 10 papillomatous and 10 nonpapillomatous suckers were lightly anaesthetized with MS–222 and injected subdermally into the upper lip with trout muscle extract containing the following: 4.07 ng polychlorinated biphenyls (PCBs), 2.025 ng organochlorine insecticides, and 0.6085 pg PCDDs-PCDFs in 50 μL (2:7) dichloromethane: hexane. The potent patholological and embryotoxic effects of this mixture on other fish were established in a previous study (Harris et al. 1994).

The control group of 10 papillomatous and 10 nonpapillomatous suckers received 50 μL of sunflower oil only. Injections were repeated every 14 days for 10 weeks (five injections). Experimental suckers were examined before each injection and any lip papillomas were measured, and locations were recorded as described previously. At the end of these experiments, suckers were killed by an overdose of MS–222. Samples of normal epidermal tissue and any papillomas that were present were removed and either placed in tissue cassettes and transferred to Bouin's fixative for histological analysis or frozen in liquid nitrogen for biochemical analysis.

Light and electron microscopy

Tissues were left in Bouin's fixative for a maximum of 48 h, washed under running water for 30–40 min, transferred to 70% ethanol, embedded in paraffin, cut with a microtome (5 μm), and placed on slides according to the methods described by Grimstone and Skaer (1972). Slides were dried overnight at 60°C, stained in hematoxylin and eosin, and examined by light microscopy.

Normal epidermal tissue and lip papilloma intended for examination by transmission electron microscopy were left in 3% buffered gluteraldehyde solution for at least 1 month. Following this, tissues were sectioned into cubes of approximately 1 mm^3 and placed overnight in 2.5% buffered gluteraldehyde (pH 7.2). Tissue sections were rinsed twice in Sorensen's washing buffer (pH 7.4) and post-fixed in 1.0% osmium tetroxide (pH 7.3), followed by two consecutive washings in Millonig's phosphate buffer (pH 7.3). This was followed by a series of dehydration steps with 50–100% ethanol.

Dehydrated sections were infiltrated through a graded series of propylene oxide into Epon–Araldite resin (3.0 mL Araldite, 5.0 mL Epon–812, 11.0 mL dodecyl succinic anhydride, 0.57 mL 3,4,6-tris-(dimethylaminomethyl)phenol) over 6 h. Fully infiltrated tissues were placed in BEEM tissue capsules, embedded in Epon–Araldite, and placed in a 70°C oven overnight to harden. Each block of resin was trimmed to expose a facet of tissue suitable for sectioning with glass knives into 60- to 100-nm sections and placed on copper grids. All sections were stained with lead citrate and 3.0% uranyl acetate, then rinsed with distilled water before being examined on a Zeiss EM 9S2 electron microscope.

Biochemical analysis

Protein kinase C (PKC) activity was assayed as described previously (Premdas and Metcalfe 1994; Premdas et al. 1995). Briefly, approximately 1.0 g of frozen tissue was thawed, weighed, and homogenized on ice using a Potter–Elvheljm tissue grinder. PKC enzyme activity was determined using the Amersham PKC assay kit (Amersham RPN–77), which is based upon the PKC-catalyzed transfer of the γ-phosphate group of adenosine-5′-triphosphate to a peptide specific for PKC. A unit of PKC activity was defined as the amount of enzyme that would transfer 1 pmol phosphate/min to its substrate under optimal conditions. A two-tailed t test ($p < 0.05$) was used to test for the significance of differences in mean PKC activity between groups.

For the determination of ornithine decarboxylase (ODC) activity, frozen epidermal tissues and papilloma samples separate from those analyzed for PKC activity were thawed, weighed, and placed in ice-cold homogenizing buffer (0.1 mM ethylenediaminetetraacetic acid (EDTA), 2.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonylfluoride in 25 mM Tris-HCl, pH 7.5) at a ratio of 1.5 mL buffer/g of tissue and homogenized as described previously. Homogenates were centrifuged (15 000 × g at 4°C for 25 min), and the supernatants were flash frozen in liquid nitrogen and immediately transferred to a −80°C freezer where they were stored for a maximum of 2 months before being assayed for ODC activity.

ODC activity was measured as the liberation of $^{14}CO_2$ from L-[1-^{14}C]ornithine (Potter et al. 1982). To measure enzyme activity, 50 μL supernatant was added to 200 μL assay medium containing 625 μM ornithine, 200 μL pyridoxal-5′-phosphate, 2.5 mM dithiothreitol, 1.0 mM EDTA, and 3.7×10^3 Bq L-[1-^{14}C]ornithine (specific activity 18.5×10^8 Bq/mM) in 12.5 Tris-HCl, pH 7.3 (Koza et al. 1991). The mixture was incubated at 37°C (Corti et al. 1987; Koza et al. 1993) for 30 min in a 2-mL Eppendorf microcentrifuge tube, checked by the addition of 500 μL 1 M citric acid, and allowed to stand at the same temperature for a further 20 min. During this time $^{14}CO_2$ liberated from the reaction was trapped on a 3-mm Whatman No. 1 filter that was saturated with 20 μL 2 M NaOH and placed in the cap of the closed microcentrifuge tube. Filters were then placed in 20-mL borosilicate vials and counted for ^{14}C in 10 mL CytoStint ES™ scintillation cocktail (ICN Biomedicals, Costa Mesa, Calif.) using a Beckman liquid scintillation counter, model LS 1701 (Beckman Instruments, Fullerton, Calif.).

To assay for pyridoxal-5′-phosphate (PLP) dependence of ODC activity, supernatants were incubated in the above medium without PLP (Koza et al. 1993). To test whether a guanosine-5′-triphosphate (GTP)-dependent form of ODC was present, papilloma and epidermal supernatants were incubated in the whole reaction mixture supplemented with 0.1 mM GTP (Koza et al. 1991). Blanks contained 50 μL homogenizing medium only. A unit of ODC activity is expressed as nanomoles of $^{14}CO_2$ evolved per hour at 37°C per milligram of protein. Supernatant protein concentrations were measured using the Bio-Rad protein assay system (Bio-Rad) using bovine

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serum albumin as a standard. A two-tailed t test ($p < 0.05$) was used to test for the significance of differences in mean ODC activity between experimental groups.

Results

Papilloma transmission studies

In the horizontal transmission experiment, 5 of 20 Squaw suckers developed small lip hyperplasia after 12 weeks, and there were 7 mortalities in the experimental group of Ganaraska suckers. Approximately 30% (7 of 21) of surviving Ganaraska suckers not initially affected with papilloma in the control A treatment developed true papillomas over the same period. However, no nonpapillomatous Squaw suckers in the control B treatment developed hyperplastic lip lesions independently. During the horizontal transmission experiments there were four deaths in each of the control A and control B groups.

Lip papillomas in captive Ganaraska suckers used in the experimental group were similar in gross appearance to lip papillomas described previously in wild Ganaraska suckers (Premdas et al. 1995). Papillomas were raised, nonpalpable nodules (2–35 mm^2) that were continuous with surrounding lip tissue. Morphologically, papillomas ranged from discrete foci of raised tissue to exfoliate cauliflower-like growths. Lip lesions that developed with time in Squaw suckers from the experimental group were visible as small areas of epithelial hyperplasia (2–5 mm^2) and did not grow to the same size as those initially present on Ganaraska suckers in the same tank. In gross appearance, the smaller lip lesions from this group were transparent or very pale with a texture similar to that of normal epidermis and generally located on the upper lip. No local inflammation was associated with these hyperplastic lesions. In contrast, lip papillomas that developed on Ganaraska suckers from the control A group were morphologically similar to papillomas initially present on Ganaraska suckers from the experimental group. No body papillomas were observed in this study.

In the cell-free filtrate experiment with filtrate prepared from wild Ganaraska River suckers, 3 of 18 surviving suckers developed lip papillomas (Table 1). However, in the treatment with filtrate prepared from proliferating papillomas from captive Ganaraska suckers, 21 of 40 surviving fish developed lip papillomas (Table 1). No animals injected with filtrate from trout muscle developed papillomas or hyperplasias of any kind and only 1 of 35 control suckers developed a papilloma. Papillomas that developed over 12 weeks on Squaw suckers following injections with proliferating papilloma filtrate were similar in gross appearance and size to papillomas taken from Ganaraska River suckers. During the experiments, there were 18 deaths (10 suckers injected with proliferating papilloma filtrate, 7 suckers injected with Ganaraska River papilloma filtrate, 1 sucker injected with trout muscle filtrate, and no suckers injected with homogenizing medium). Although all new papillomas developed on the lip surface, only two papillomas developed at the actual site of injection.

In the contaminant injection study, no nonpapillomatous Ganaraska suckers developed papillomas after 10 weeks. In this study, only one initially papillomatous sucker exhibited papilloma growth and one initially nonpapillomatous sucker died. Papilloma regression of between 30 and 80% of initial papilloma surface area was observed in all remaining animals. In control groups injected with sunflower oil, no nonpapillomatous suckers developed papillomas, and papilloma regression of between 40 and 100% of initial papilloma surface area was observed in all initially papillomatous suckers. During these experiments, two nonpapillomatous suckers and two papillomatous suckers injected with sunflower oil died.

Table 1. Cell-free filtrate study: incidence of papilloma at week 0 (no papillomas) and week 12 for Squaw suckers exposed to fortnightly subdermal injections (20 µL) of cell-free filtrate prepared from wild Ganaraska papilloma (A), captive proliferating Ganaraska papilloma (B), and trout muscle tissue (C) and controls (D) injected with 20 µL of homogenizing medium (one part DMSO in four parts phosphate-buffered saline).

	n			
		Week 12		
Filtrate	Week 0 (no papillomas)	Deaths	No papillomas	Developed papillomas
A	25	7	15	3
B	50	10	19	21
C	17	1	16	1
D	35	0	34	1

Light microscopy

In normal lip tissue from white suckers (Fig. 1*a*), the top three to seven cell layers of the epidermis are composed of flattened squamous epithelial cells. The underlying malphigian cells are cuboidal and well organized into a layer approximately 15–50 cells thick. Numerous cellular structures described as chemoreceptors, possibly the equivalent of mammalian taste buds, are randomly distributed throughout this layer and in some cases open to the outside. The generative cell layer is organized in a single stratum of darkly staining columnar cells perpendicular to, and resting on, a continuous basal lamina. The basal lamina is in turn underlayed by a poorly organized collagenous dermis and some cartilage constituting parts of the jaw. Areas of hyperplasia that developed on the lips of Squaw suckers in the experimental group of the horizontal transmission study were only mildly hyperplastic with malphigian layers 30–100 cells thick. No compression of epithelial chemoreceptors was observed and only low numbers of inflammatory infiltrates were present in these hyperplastic lesions.

Papillomas from wild Ganaraska suckers (Fig. 1*b*) and papillomas induced in Squaw fish by injection of papilloma filtrate (Fig. 1*d*) did not differ histologically. These papillomas induced in Squaw suckers were also histologically similar to papillomas described for wild suckers from other areas of the Great Lakes (Smith et al. 1989*a*, 1989*b*). Compared with normal tissue, the top layer of papilloma epidermis was flattened and between three and five cells thick, although in some cases, this superficial epidermal layer was not present. The underlying malphigian cells were cuboidal but smaller and more tightly packed than in normal tissue, and this layer was up to 200–300 cells thick with frequent mitotic figures, indicating increased cellular proliferation. Chemoreceptor structures were either absent or compressed by surrounding hyperplastic tissue (Fig. 1*c*). Inflammatory cells in these tumours consisted mainly of granulocytes and occasional macrophages. In larger

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Fig. 1. Light micrographs of lip tissue and lip papillomas. (*a–c*) Wild Ganaraska white suckers. (*a*) Transverse section of normal lip tissue. A well-organized dermis (D), an epithelium comprised of large malphigian cells (M), and numerous chemoreceptors (C) are present. (*b*) Cross section of benign lip papilloma. The dermis (D) is markedly compressed by proliferating, but noninvasive, malphigian cells (M). Note the absence of chemoreceptors. (*c*) Cross section of a large lip papilloma. The proliferating malphigian cells (M) have not invaded the dermis (D), indicating that this is a benign epithelial tumour. There is some dermal retraction as a result of fixation. (*d*) Transverse section of a filtrate-induced lip papilloma. Note the similarity to wild Ganaraska (donor) papilloma; the dermis (D) is compressed by proliferating, but noninvasive, malphigian cells (M). No chemoreceptors are present. Scale bars = 300 μm.

Ganaraska papillomas, epidermal pegs consisting of malphigian cells were observed that extended into the dermal layer.

In the cell-free filtrate experiment, injection of homogenizing medium (PBS–DMSO) did not provoke a proliferative or inflammatory response, and after 12 weeks, lip tissue treated with PBS–DMSO was histologically and morphologically indistinguishable from untreated lip tissue. Injection of trout cell-free filtrate produced mild inflammation at the injection site but no hyperplasia. In the epidermis, structures resembling granuloma were observed that surrounded the site of injection. No areas of necrosis or hemorrhage were observed, and cellular architecture was otherwise histologically similar to that in untreated and control tissue. In contaminant injection studies, minor necrosis and hemorrhage were occasionally observed at the injection sites but no hyperplasia developed as a result of these injections. Apart from these localized reactions, contaminant-injected normal lip was histologically and morphologically indistinguishable from untreated lip tissue. After 12 weeks, regressing papillomas resembled the mild hyperplasia observed on suckers in the horizontal transmission study. The malphigian layer of these hyperplastic areas was less than 100 cells thick, there was no compression of chemoreceptors by surrounding epithelial cells, and few inflammatory cells were observed.

Transmission electron microscopy

Transmission electron microscopy of malphigian and epithelial cells in normal tissue revealed typical eukaryotic organization with a well-defined nucleus and extensive endoplasmic reticulum and Golgi apparatus. Some tonofilaments transversed the cells and apparently passed through desmosomes at the transitional cell membrane – intracellular space zone. Desmosomes were uniformly distributed between the intracellular spaces surrounding the cells and were usually located at the fringe of blunt extensions of the plasma membrane. Intracellular spaces were uniform in size between malphigian cells but smaller and more variable in the squamous epithelial cells. No viruses were observed in normal epidermal tissue taken from either Squaw or Ganaraska River fish. Similar findings were reported by Smith et al. (1989*a*).

The ultrastructural organization of wild and induced papilloma was similar to that observed in normal epidermis. Endoplasmic reticulum was not observed in some malpighian cells, although the incidence of such cells was variable. Intracellular spaces were larger and more irregularly shaped, as compared with normal epithelium, and in some cases they were filled with what appeared to be granular material. This was more pronounced in the squamous epithelial cells, where some cell vacuolation and frequent sloughing of the uppermost cells was observed. Desmosomes were readily identifiable in the intracellular spaces, but these structures were irregularly spaced in contrast to normal epidermis. Aberrant desmosomal aggregates were observed in the transitional cell membrane – intercellular space region of some cells. These structures were previously reported to be localized in the uppermost cell layers of epidermal papilloma (Smith et al. 1989*b*). No viruses were observed in the nucleus, cytoplasm, cytoplasmic membrane, or intercellular spaces of any wild, proliferating, or induced epithelial tumours examined in this study (Figs. 2*a* and 2*b*).

Enzyme analysis

PKC activity was significantly lower in papilloma taken from wild Ganaraska River suckers than in normal epidermal tissues from Squaw and Ganaraska River suckers (Fig. 3). These results are in agreement with data reported previously for Ganaraska suckers (Premdas and Metcalfe 1994; Premdas et al. 1995). PKC activity was reduced in Squaw sucker papilloma induced from filtrates prepared from proliferating papillomas and from wild papilloma, although fewer papillomas developed as a result of the latter treatment than the former. PKC activity in proliferating papillomas was not significantly different from that measured in wild Ganaraska papilloma. However, enzyme activities were significantly lower than in normal epidermal tissue. In contrast, PKC activity in sucker epidermis injected with trout filtrate was not significantly different from that in normal tissue and was significantly greater than that in papillomas induced by injection of filtrates. There was no significant difference in PKC activity between contaminant-injected and sham-injected epidermis or between contaminant-injected and sham-injected papilloma (Fig. 4).

ODC activity in white sucker epidermis and papilloma was PLP dependent (Fig. 5*a*). ODC activity in epidermal papilloma and filtrate-induced epidermal papilloma was stimulated by the addition of GTP, but ODC activity in normal epidermis was not affected (Fig. 5*a*). These findings are in agreement with previous studies on ODC activity in neoplasms of mice and winter flounder (*Pleuronectes americanus*) (Corti et al. 1987; Koza et al. 1991, 1992, 1993). ODC activity was significantly higher in sucker papilloma than in sucker epidermis. There was no significant difference in ODC activity between contaminant-injected and sham-injected epidermis or between contaminant-injected and sham-injected papilloma (Fig. 6). However, wild papilloma and wild epidermis exhibited significantly higher levels of ODC activity than filtrate-induced papilloma or epidermis (Figs. 5*a* and 5*b*).

Discussion

In earlier studies on the Lake Ontario population of white sucker spawning in the Ganaraska River, there was an elevated prevalence of external neoplasms (46%) compared with suckers from the Squaw River (<5%). Analysis of suckers from the Ganaraska River revealed high levels of PCBs and organochlorines, while suckers sampled from the inland reference site exhibited significantly lower levels of these contaminants

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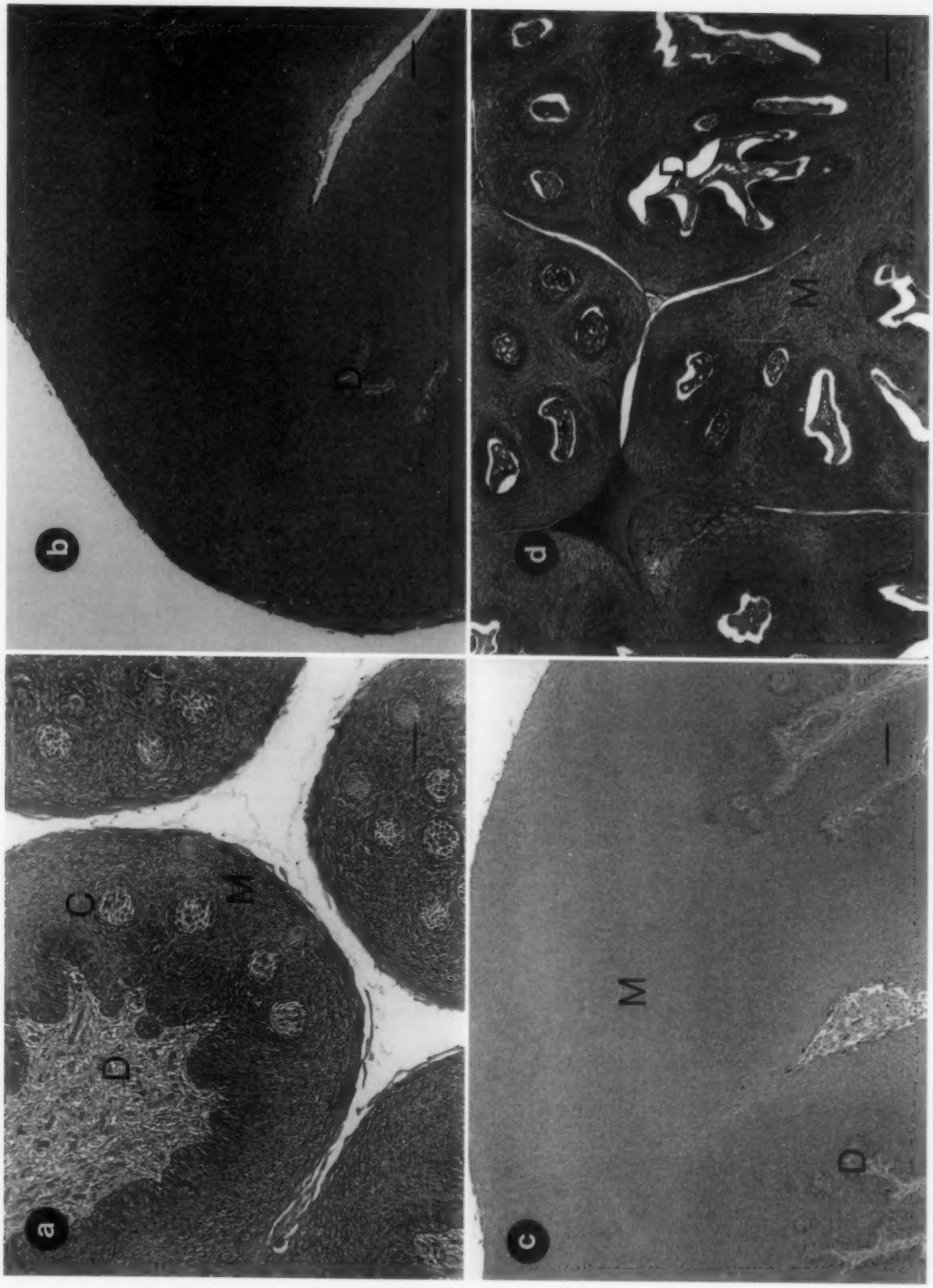

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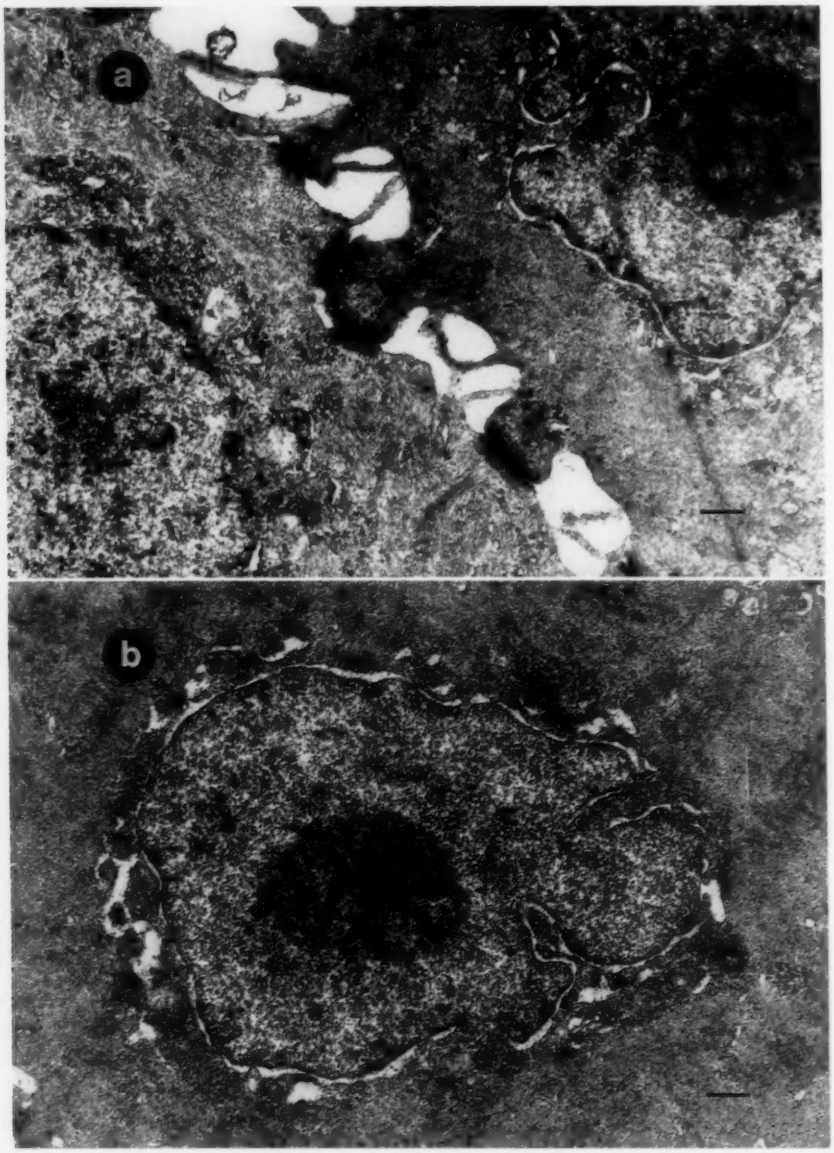
Fig. 2. Transmission electron (TEM) micrographs of malphigian cells (*a*) in the superficial layer of a filtrate-induced lip papilloma. No viral particles are visible in either the cytoplasm or intracellular spaces. (*b*) A nucleus in a filtrate-induced lip papilloma. No viral particles are visible in the nucleus or surrounding cytoplasm. Scale bars = 10 μm.



(Premdas et al. 1995). The results of the present study indicate that contaminants present in Great Lakes fish may not be the primary etiological agent responsible for papilloma development on white sucker.

Although small areas of hyperplasia were induced in Squaw River suckers by exposure to papillomatous suckers in the horizontal transmission study, these lesions did not resemble in appearance or biochemical activity the papillomas in wild fish. The origin of these lesions cannot be determined at this time. It is also unclear why ~30% (7 of 21) of surviving Ganaraska fish in the horizontal transmission control study developed papilloma, whereas no papilloma developed in the control group from the contaminant injection study. It is possible that the former group was accidentally infected by the use of improperly sterilized nets or equipment.

Injections of extracts containing organic contaminants were unsuccessful in inducing new papilloma or increasing the growth of existing papilloma. The induction of lip papilloma in suckers from injection of tumour filtrates strongly suggests a viral etiology. The presence of virus particles in papilloma

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Fig. 3. Mean (±SD) PKC activity ($n = 4$) in Ganaraska papilloma, Ganaraska normal epidermis, and Squaw normal epidermis compared with papilloma on Squaw fish induced by proliferating papilloma cell-free filtrates and lip tissue from Squaw suckers injected with trout muscle cell-free filtrates and wild papilloma cell-free filtrates.

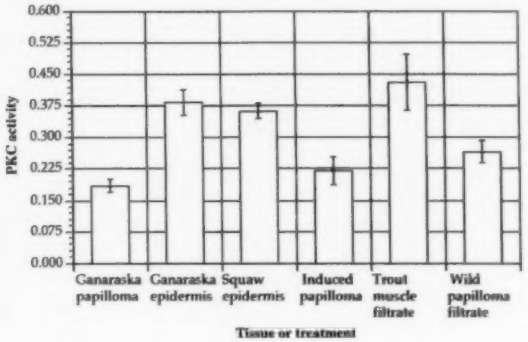
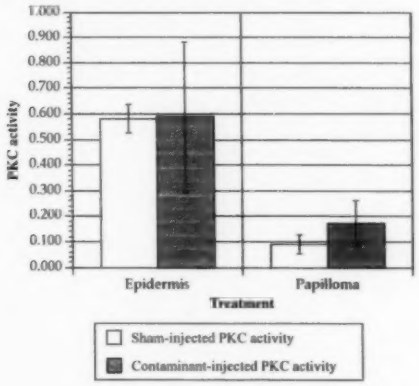


Fig. 4. Mean (±SD) PKC activity ($n = 5$) in lip tissue from nonpapillomatous and papillomatous Ganaraska suckers injected with either sunflower oil (sham) or contaminant extract.



tissue would have confirmed these observations, but viruses were not observed by transmission electron microscopy. Retrovirus particles were observed in some previous studies (Sonstegard 1977) but not in others (Smith et al. 1989*a*, 1989*b*).

No nonpapillomatous Squaw suckers (control B) spontaneously developed papilloma during this study, but approximately 40% of initially nonpapillomatous Ganaraska suckers (control A) developed papilloma when these animals were held under crowded tank conditions. These results could indicate horizontal transmission of the disease, but it is more likely that there was expression of a latent disease only in Ganaraska fish. Previous studies with Ganaraska suckers held in the laboratory under crowded conditions have shown that these fish develop papilloma through stress-related mechanisms (Premdas and Metcalfe 1994). It is also possible that Lake Ontario suckers are more immunologically (Anderson et al. 1984; Anderson 1990) or genetically susceptible to the development of spontaneous tumors compared with the inland population or that

Fig. 5. Mean (±SD) ODC activity ($n = 3$) in the presence or absence of (*a*) pyridoxal-5′-phosphate (PLP) or (*b*) guanosine-5′-triphosphate (GTP) in normal lip tissue and filtrate-induced lip papilloma in Squaw suckers, compared with wild Ganaraska papilloma.

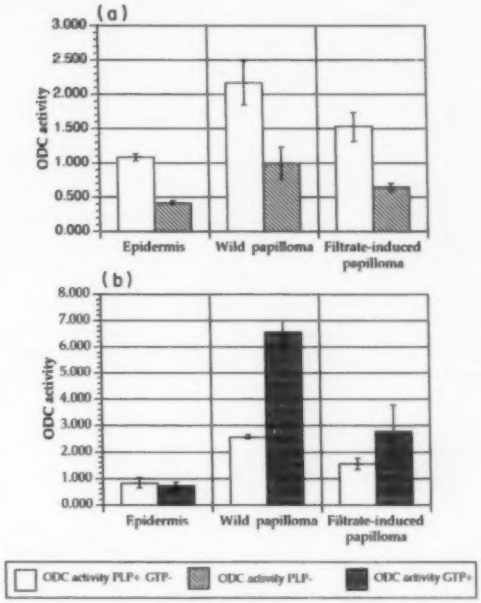
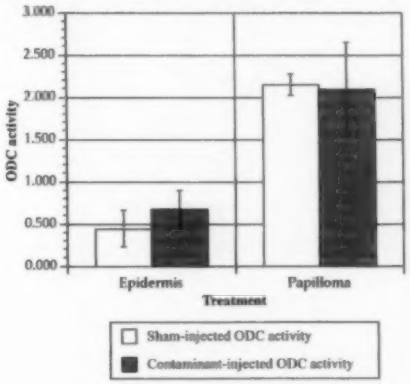


Fig. 6. Mean (±SD) ODC activity ($n = 5$) in lip tissue from nonpapillomatous and papillomatous Ganaraska suckers injected with either sunflower oil (sham) or contaminant extract.



skin cells in Lake Ontario suckers were already chemically initiated.

Previous attempts to induce papillomas using cell-free filtrates were unsuccessful (Sonstegard 1977), and the low rate of induction (3 of 18) in our experiments with filtrates prepared from wild fish appears to support these earlier observations. However, when filtrates prepared from proliferating Ganaraska papillomas were injected into Squaw suckers, approximately half (21 of 40) of these animals developed lip papillomas. Suckers injected with trout serum did not develop

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papillomas and there was only one incident (1 of 35) of papilloma development in suckers injected with homogenizing medium. These data suggest that these lesions did not develop from an immune reaction or from injection-related stress. We presently have no explanation as to why these lip papillomas very rarely developed at the injection sites. However, subdermal injections would have delivered filtrates into the collagenous dermis that underlies the entire lip (Smith et al. 1989*a*, 1989*b*; Premdas et al. 1995), so it is possible that lip papillomas developed at small preinitiated sites not visible under light microscopy.

Although the exact mechanisms involved in the transmission of papilloma are unclear, several hypotheses can be formulated. Perhaps a papillovirus in white sucker is expressed only in proliferating tumours undergoing rapid growth, or alternatively, the data could indicate that the virus is infective only under very specific conditions. It has been suggested that papilloma in wild fish may develop in response to a virus that is activated by long-term exposure to chemical pollutants (Black 1983, 1984). It is possible that proliferating papillomas taken from Ganaraska suckers held in the laboratory carried chemically activated papilloviruses. This does not explain why contaminant injections were unsuccessful in inducing new papillomas or increasing the growth of existing papillomas.

Our data support a hypothesis for a multifactoral etiology of papilloma development on suckers. Filtrates prepared from wild Ganaraska papillomas were less successful in inducing tumours. It is likely that neoplastic tissues in wild suckers advance and regress in response to a variety of environmental stimuli and that cells from regressive and proliferative tumours are very different biochemically and physiologically. For instance, previous studies have shown that regressing papillomas exhibit high levels of PKC activity comparable with normal epidermis, but PKC activity is significantly reduced in proliferating papillomas (Premdas and Metcalfe 1994). If the wild papillomas used in this study were not in a proliferative state at the time of sampling this may explain why filtrates prepared from these tumours were unsuccessful in inducing papilloma development.

PKC is a rate-limiting cellular enzyme implicated in transmembrane signaling and regulation of the cell cycle (Rosner et al. 1985; Wolf et al. 1985; Nishizuka 1988). In hyperplasia and neoplasia or in tissues where hyperplastic or neoplastic transformation has been initiated, cytoplasmic PKC activity declines or becomes down regulated as it binds to and phosphorylates membrane-bound regulatory proteins, e.g., epidermal growth factor (Stoschek and King 1986; Newsted and Giesy 1992). In addition, PKC appears to regulate inositol phospholipid cycling (Leeb-Lundberg et al. 1985; Connolly et al. 1986) and may modulate growth factor receptor responsiveness on cell membranes (Jacobs et al. 1983; Klausner et al. 1984). Thus, downregulation of cytoplasmic PKC may release cells from normal growth-inhibitory controls and may be a permissive event for a proliferative response (Hansen et al. 1990). In higher vertebrates, reduced PKC activity is correlated with hyperplasia and neoplasia (Fischer et al. 1989; Hansen et al. 1990; Droms and Anderson 1991; Brooks et al. 1991). In suckers, the low levels of PKC activity observed in lip papillomas appear to corroborate these earlier mammalian studies (Premdas and Metcalfe 1994).

ODC is an inducible, rate-limiting enzyme associated with cell cycle control, and enzyme activity is invariably elevated in proliferative tissues such as neoplasms (Snyder and Russel 1970; Janne et al. 1978). Putrescine (diamine) and spermidine and spermine (polyamines) are aliphatic nitrogenous bases necessary for the synthesis, structure, and function of DNA, RNA, and cellular proteins (Baserga 1985). Increased diamine and polyamine production is an obligatory event in the induction of cell division or proliferation, and the induction of ODC activity in cells is linked to a concurrent increase in the production of these compounds (Heby and Janne 1981; Takigawa et al. 1983). However, the exact mechanism linking these two inducible processes has not been clearly delineated (Weeks et al. 1982; Takigawa et al. 1982, 1983; Hibasami et al. 1988).

In earlier mammalian studies, the levels of ODC activity in normal epidermis were found to be quite low. In contrast, epidermal tumours expressed significantly higher levels of ODC and inducible ODC activity (Astrup and Boutwell 1982; O'Brien 1976; O'Brien et al. 1975). Our results appear to corroborate these earlier findings in that ODC activity in white sucker papillomas was significantly higher than ODC activity in normal sucker epidermis. Like mammalian tumours, sucker papillomas contained a functionally distinct (GTP and PLP dependent) form of ODC (O'Brien et al. 1986, 1987).

There is some evidence that ODC induction may be mediated by PKC (Verma et al. 1986) and that in proliferating epidermal cells, PKC and ODC are differentially regulated (Fischer et al. 1989; Hansen et al. 1990). In epidermal hyperplasia induced by TPA (12-*O*-tetradeconylphorbol-13-acetate) in mice, down regulation of PKC is synchronous with ODC induction (Mills and Smart 1989; Fischer et al. 1989). It is suggested that differential activations of these two enzymes are permissive events for epidermal hyperplasia and tumour promotion (Hansen et al. 1990). Because increased levels of ODC were correlated with reduced PKC activity in wild and filtrate-induced sucker papilloma, there is some indication that similar mechanisms may be involved in papilloma development in fish.

Our data suggest that PKC and ODC may be good marker enzymes for distinguishing papilloma from normal tissue and that enzyme activity indicates the state of papilloma development in white sucker. In some cases, PKC and ODC activity studies may be used to corroborate evaluations primarily based on histological determination. ODC and PKC activity were altered in both wild and induced papilloma in comparison with normal sucker epidermis. There was no significant difference in ODC activity between contaminant-injected and sham-injected epidermis or between contaminant-injected and sham-injected papilloma. However, wild papilloma and wild epidermis exhibited significantly higher levels of ODC activity than filtrate-induced papilloma and sham-injected epidermis. Although ODC activity is increased in tumors and neoplasms compared with normal tissue (Astrup and Boutwell 1982), the enzyme may be subjected to regulated degradation in mitogenically stimulated cells (Hayashi and Murakami 1995) possibly to prevent cellular cytotoxicity owing to polyamine overproduction (Morris 1991). This might help explain why lower levels of ODC activity were observed in the rapidly growing filtrate-induced papilloma than in the wild papilloma.

Papillomatosis is expressed only in a small proportion of suckers from unpolluted regions, whereas in polluted environments there is a higher prevalence of this disease (Smith et al.

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1989*a*, 1989*b*; Premdas et al. 1995). A virus appears to be the primary etiologic agent for these neoplasms but expression of the disease could be influenced by such factors as (*i*) long-term exposure to chemicals resulting in immune deficiency (Anderson 1990) or other sublethal toxic effects, (*ii*) crowding during migratory spawning (Premdas and Metcalfe 1994), (*iii*) concurrent disease, as in hepatic neoplasms (Kirby et al. 1990; Hayes et al. 1990), or (*iv*) hormonal status. Other environmental stressors such as habitat alterations, eutrophication, overfishing, introduction of exotic species, and even disturbance from passing vessels could also adversely affect fish health. Nonanthropogenic stressors, such as naturally occurring toxins and carcinogens, poor water quality, or temperature fluctuations, are also increased in polluted environments (Kurelec et al. 1981), and these factors may also influence fish health. The effects of nonchemical stresses on fish populations are often indistinguishable from contaminant effects (Munkittrick and Dixon 1989). Thus, the etiology of epidermal papillomas in white sucker may be a multifactoral one in which the initiation and development of virally induced papilloma is modified by a number of environmental factors.

Acknowledgments

Research was funded by a Natural Sciences and Engineering Research Council operating grant to C.D.M. We thank the following individuals who provided technical and field assistance during this study: L.E. Kimpe and D. Lietz, Department of Environmental and Resource Studies, Trent University, for the transmission electron microscopy work; E. Bennett, Department of Environmental and Resource Studies, Trent University, for field and laboratory assistance; and Dr. H.W. Ferguson, Department of Pathology, University of Guelph, for histopathological examination of white sucker tissue (1992). The assistance of the Ontario Ministry of Natural Resources and of David Bell in particular was appreciated.

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Effect of ration and seawater transfer on food consumption and growth of Atlantic salmon (*Salmo salar*) smolts

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Abstract: In Atlantic salmon smolts, fed at ration levels of 0.5, 1.0, and 3.0% body weight·day^{-1}, growth performance in fresh water was not a significant determinant of subsequent seawater growth ($P > 0.05$). X-radiography was used to measure food consumption rates of individual fish. Consumption rates of fish fed low and intermediate rations were similar in fresh water and seawater ($P > 0.05$). However, consumption rates for fish fed a high ration level were two times greater in seawater than in fresh water ($P < 0.05$), possibly owing to higher temperatures. Specific growth rates were generally negative in the period immediately following transfer to seawater and food consumption rates were lower than in fresh water for fish on high ration levels. Specific growth rates increased with increasing ration level in both fresh water and seawater. A linear regression model gave the best description for the relationship between individual specific growth rates and food consumption for all ration groups in both fresh water and seawater. Slopes and intercepts for the low ration groups in fresh water were similar to values for the same fish in seawater ($P > 0.05$). The intermediate and high ration groups had steeper slopes in seawater than in fresh water ($P < 0.001$).

Résumé : Chez les smolts de saumon de l'Atlantique auxquels on a administré des rations alimentaires correspondant à 0,5, 1,0 et 3,0 % de leur poids corporel par jour, la performance de la croissance en eau douce n'était pas un paramètre important de leur croissance ultérieure en eau salée ($P > 0,05$). On a utilisé des radiographies pour mesurer le taux de consommation alimentaire de chaque poisson. Le taux de consommation des poissons recevant des rations faibles et intermédiaires était similaire en eau douce et en eau salée ($P > 0,05$). Toutefois, celui des poissons recevant une ration élevée était deux fois plus grand en eau salée qu'en eau douce ($P < 0,05$), ce qui s'explique probablement par des températures plus élevées. En général, les taux de croissance particuliers étaient négatifs dans la période qui suivait immédiatement le passage en eau salée et le taux de consommation des aliments était plus faible qu'en eau douce pour les poissons recevant une ration élevée. Les taux de croissance particuliers augmentaient avec l'augmentation de la ration en eau douce et en eau salée. C'est un modèle de régression linéaire qui décrivait le mieux la relation entre le taux de croissance de chaque spécimen et la consommation d'aliment pour tous les groupes de ration, tant en eau douce qu'en eau salée. Les pentes et les coordonnées à l'origine pour les groupes à qui on a administré une ration faible en eau douce étaient similaires aux valeurs pour les mêmes poissons en eau salée ($P > 0,05$). Dans le cas des groupes recevant une ration intermédiaire et élevée, les pentes étaient plus abruptes en eau salée par comparaison avec l'eau douce ($P < 0,001$).
[Traduit par la Rédaction]

Introduction

A critical phase in the life cycle of Atlantic salmon, *Salmo salar* L., is the parr smolt transformation, which involves a number of behavioural, morphological, and physiological changes. Some of these changes are regarded as pre-adaptive, others as being contingent on transfer to seawater (McCormick and Saunders 1987; Hoar 1988). These complex changes are affected by many abiotic and biotic factors (Brett 1979). In culture, transfer of smolts from fresh water to seawater is often associated with high mortalities (Johnstone 1990), and a knowledge of the factors that affect growth and survival after transfer to seawater is therefore desirable to commercial fish farmers.

Among salmonids, relative growth performance in fresh water and seawater varies between species and is also influenced by fish size, feeding, and other environmental variables, particularly temperature (Elliott 1982). MacLeod (1977) reported a decrease in food intake and growth for age 0+ rainbow trout, *Oncorhynchus mykiss*, acclimatized to seawater for 14 days. Shaw et al. (1975*a*), however, found that the growth rates of 21-month-old Atlantic salmon parr at a range of salinities were similar to those of fish retained in fresh water. In groups of Atlantic salmon smolts acclimatized to three salinities and four different ration levels, McCormick et al. (1989) found ration to be more important than salinity in affecting growth rates. Usher et al. (1991) concluded that temperature exerted a far greater influence on growth in smolts than salinity. Smith and Thorpe (1976) found that the growth rates of seawater-adapted yearling trout varied seasonally and with ration level, but exceeded those of sibling fish in fresh water

Received July 5, 1995. Accepted November 11, 1995.
J12983

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when compared over 1 year. Clearly these findings reflect differences in experimental approach and the time over which comparisons are made. It is, however, evident that the effect of seawater transfer on growth will be difficult to determine without specific knowledge of food consumption.

X-radiography (Talbot and Higgins 1983) has been used in a number of studies to measure consumption rates of individual salmonids held in groups (Jobling et al. 1989; Jørgensen et al. 1993; McCarthy et al. 1993) and more recently to study feeding in exercised and unexercised Atlantic salmon before and after transfer to seawater (Jørgensen and Jobling 1994). The latter authors found that both food intake and growth rates were reduced for up to 14 days following seawater transfer but after 28 days they returned to values comparable to those observed in fresh water. Apart from this study, there is little information on the relationship between consumption and growth in the periods before and after seawater transfer in Atlantic salmon and no studies have followed the same identified individuals over this period. In the present study, X-radiography was used to measure individual food consumption rates in Atlantic salmon smolts in the period immediately before and after transfer to seawater. Relationships between consumption and growth, food conversion efficiencies, and the effect of transfer on growth performance were studied in fish fed at three different ration levels.

Materials and methods

Maintenance and feeding of fish

Atlantic salmon presmolts derived from mixed-parentage Scottish farmed stocks were maintained at the Scottish Office, Agriculture, Environment and Fisheries Department (SOAEFD) experimental facility at Aultbea, Wester Ross, Scotland, in circular stock tanks (1 m diameter, 400 L) supplied with oxygen-saturated fresh water at a rate of 10 L·min^{-1}. Prior to the beginning of the experiment, fish had been fed commercial pelleted Royale Freshwater Smolt (BP Nutrition UK Ltd.) diet at 2.0% body weight·day^{-1} from 30 November 1992 until 29 February 1993, increasing to 3.0% body weight·day^{-1} thereafter. Food was delivered in discontinuous 15-min pulses throughout daylight hours, using an automatic disc feeder.

On 19 March 1993, 240 fish were anaesthetized (MS–222, Sigma; 0.1 g·L^{-1}), weighed (initial wet weight 30.0 ± 2.0 g), and randomly allocated to one of six tanks, identical to the stock tanks. Initially, each tank contained 40 fish that were individually identified by a unique combination of Alcian blue marks on the ventral surface. The tanks were assigned to one of three groups and fish in replicate tanks were fed a low, medium, or high ration (0.5, 1.0, and 3.0% body weight·day^{-1}, respectively). Feed was delivered over a 2-h period between 11:00 and 13:00.

Following an initial acclimation period (19 March – 13 April 1993), fork lengths, wet weights, and food consumption rates were measured for all individual fish on four occasions in fresh water between 14 April and 15 May 1993. On 16 May fresh water was replaced by seawater, which was supplied at 10 L·min^{-1} until the end of the experiment in August 1993. The total amount of food delivered to each group was adjusted after each sampling occasion as a calculated percentage of the total wet body weight for each tank of fish.

Fish were maintained under natural photoperiod, which varied between 10 and 15 h of daylight through the course of the study. The experiment was conducted under ambient freshwater (mean 8.8°C, range 6–14°C) and seawater (mean 12.9°C, range 10–15°C) temperatures.

Mortality rates after recovery from anaesthesia were high in one of the intermediate ration groups (1% body weight·day^{-1}) at an early stage of the study and data from this group were excluded from statistical analyses. Failure of the seawater supply resulted in 100% mortality in one of the high ration groups (3% body weight·day^{-1}) on 29 June 1993. However, overall sufficient fish survived for up to 12 weeks after transfer for meaningful comparisons of differential performance to be made for all ration levels.

Measurement of individual food consumption

Food consumption of individual fish was monitored on four occasions in fresh water and on two occasions after fish had been transferred to seawater, using a modification of the X-radiography technique described by Talbot and Higgins (1983). On the day that consumption rates were determined, the fish were fed their usual diet, repelleted after the addition of ballotini glass spheres (400–520 μm, 2% wet weight, Jencons Scientific Ltd). The repelleted diet was prepared using the method of McCarthy et al. (1992) and fed to the fish at the same ration level and for the same duration as the normal diet. One hour after feeding had ceased, fish were anaesthetized then X-rayed in groups of 10 using a portable X-ray machine (Todd Research model 20/80) and Kodak Industrex CX film. An experiment on gastric emptying was performed to ensure that the 2-h feeding period in this study was suitable for measuring food consumption using X-radiography under experimental conditions. Random samples of 10 individuals were selected from each of the six tanks and X-rayed at 30-min intervals between 1 and 6 h after each of the feeding periods. Examination of the X-radiographs revealed that the number of ballotini glass spheres in the alimentary tract of individual fish remained constant, suggesting that consumption could be accurately estimated if fish were X-rayed within 6 h of the end of the feeding period.

On each sampling occasion wet weights (in grams) and fork lengths (in millmetres) were recorded and fish were returned to the tank. The number of ballotini glass spheres in the alimentary tract of each fish was counted from the X-ray negatives.

χ^2 goodness of fit tests were performed for labelled subsamples of repelleted food over a range of weights (0.05–10.0 mg dry food) and the number of ballotini glass spheres in any given sample of food was shown to be Poisson distributed ($P > 0.05$). Pearson standardized residuals were calculated against the expected number of ballotini glass spheres as follows: standardized resdidual = $(O - E)/E^{½}$, where O and E are the observed and expected number of ballotini glass spheres. Examination of the standard residuals against the expected number of ballotini glass spheres showed that there was no pattern in the effects and confirmed that the number of ballotini glass spheres in a sample of food fitted the Poisson distribution, suggesting that they were completely mixed throughout the food. The expected weight of food per ballotini glass sphere was calculated using a point estimate: 1 ballotini glass sphere = Σ weight / Σ ballotini glass spheres, where Σ weight represents the sum of labelled subsamples of repelleted food (milligrams of dry food) and Σ ballotini glass spheres is the total number of ballotini glass spheres in the previously weighed subsamples. The weight of fish was corrected for the weight of food in the gut by subtracting the dry weight of food consumed by individual fish from the dry weight of fish and expressed as mg dry food·g dry weight fish^{-1}·day^{-1} (McCarthy et al. 1993). Occasionally fish moved during the X-ray exposure and blurred images made it difficult to count ballotini glass spheres accurately. Data from these fish were excluded from the analyses. Percent moisture was determined for whole bodies of frozen fish, freeze dried to a constant weight. The mean percent moisture content for a control group of fish ($N = 15$) in fresh water was determined as 73.3 ± 0.2% and was used to convert wet weights to dry weights; no significant differences were observed between ration levels. Percent moisture content for fish in seawater ($N = 45$) at different ration levels was calculated to be: 0.5%, 77.8 ± 0.3%; 1.0%, 76.5 ± 0.3%; and 3.0% body weight·day^{-1}, 74.6 ± 0.2%; significant differences were observed for groups of fish between ration levels.

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Table 1. Effects of ration level and seawater transfer on initial and final dry weights (g), specific growth rates (SGR, % dry body weight·day^{-1}), mean consumption rates (mg dry food·g dry body weight^{-1}·day^{-1}), growth efficiencies (GE, % g dry body weight·mg dry food^{-1}), food conversion ratios (FCR, mg dry food·g wet body weight^{-1}), and condition factors (K, g·cm × 100) of individual Atlantic salmon in the freshwater phase (14 April – 15 May 1993).

	Rations (% body weight·day^{-1})				
	0.5	0.5	1.0	3.0	3.0
Number of fish					
Initial	40	40	40	40	40
Final	36	35	40	36	39
Dry weight (g)					
Initial	8.49±0.07*ab*	8.65±0.07*b*	8.27±0.07*a*	8.54±0.07*ab*	8.47±0.08*ab*
Final	9.91±0.12*a*	10.08±0.10*a*	9.86±0.13*a*	10.17±0.14*a*	10.19±0.13*a*
SGR (%·day^{-1})	0.53±0.03*a*	0.53±0.03*a*	0.60±0.02*ab*	0.60±0.04*b*	0.63±0.02*b*
Mean consumption (dry mg·g^{-1}·day^{-1})	14.37±0.68*ab*	11.66±0.65*a*	20.20±0.98*cd*	21.11±1.02*d*	17.46±0.64*bc*
GE (%)	37.69±1.79*b*	48.18±3.06*c*	30.63±1.38*ab*	28.01±1.61*a*	36.60±1.42*b*
FCR	0.77±0.04*ab*	0.66±0.06*a*	0.93±0.04*b*	0.88±0.09*ab*	0.77±0.03*ab*
K (b = 2.730)	2.12±0.02*a*	2.15±0.02*a*	2.10±0.01*a*	2.14±0.02*a*	2.17±0.01*a*

Note: Values are given as the mean ± standard error. Means followed by the same letter were not significantly different at the 5% level (Scheffé's multiple comparison test). Food consumption data are based on the mean of four sampling occasions.

To represent negative growth at zero consumption values (Carter et al. 1992*a*, 1994), two groups of fish (N = 15) were weighed, transferred to tanks identical to the experimental tanks, and starved for 14 days in either fresh water (14–28 April 1993) or seawater (13–27 July 1993). Data from these groups were combined with those from fed fish in the freshwater or seawater phase as appropriate.

Data compilation and statistical analyses

The data were compiled in three time intervals: freshwater (14 April – 15 May 1993), immediate post seawater transfer (16 May – 7 June 1993), and seawater (13 July – 10 August 1993). The freshwater phase represents the period of time that the fish spent in fresh water after a 26-day acclimatization period. The immediate post seawater transfer period monitors acclimatization of fish to seawater 22 days after transfer and the seawater phase follows the progress of the fish in seawater until 10 August 1993.

The dry weight specific growth rates, SGR (%·day^{-1}), for individual fish were calculated using the following equation (Ricker 1979): $\text{SGR} = ((\ln(W_t) - \ln(W_o)) \times 100)/t$, where W_o and W_t are the initial and final dry weights of each fish (in grams) and t is the duration in days. Growth efficiency (GE, Carter et al. 1994) was calculated as dry weight using the following equation: $\text{GE} = ((W_t - W_o) \times 100)/(\text{food consumed} \times t)$, where W_t is the dry weight (in grams) at time t, W_o is the initial dry weight (in grams), and t is the number of days between weighings. Food consumption data for fresh water are based on the mean of four X-ray measurements; one measurement was used for the immediate post seawater transfer and seawater phases.

The food conversion ratio (FCR: Carter et al. 1994), the inverse of the growth efficiency equation, was calculated using wet weights: $\text{FCR} = (\text{food consumed} \times t)/(W_t - W_o)$. Condition factors ($K$) were determined according to the formula $K = (W/L^b) \times 100$, where W is the final wet weight (in grams), L is the final fork length (in centimetres), and b is the slope coefficient of the regression of $\log_{10}$ weight on $\log_{10}$ length (LeCren 1951; Bolger and Connolly 1989).

In an assessment of X-radiography for measurement of food consumption, Jobling et al. (1993) stressed that information from a single feed estimate should not be used uncritically because day to day variations in consumption rates of individual fish have been widely reported (see review by McCarthy et al. 1993). In this study consumption rates in the immediate post seawater transfer and seawater phases were derived from one X-ray sampling occasion and Kendall's coefficient of concordance (Wilkinson 1992) was used to examine the relationship between values of food consumption determined from one X-ray and the mean of three X-rays. The results suggested that for fish on intermediate and high ration levels, values from one sampling occasion were representative of the whole period. Thus, we are confident that the X-ray technique used in the manner described gave meaningful results. However, results for fish on low rations were not found to be significant, which may be due to an increase in day to day variation in meal sizes in groups fed restricted rations (McCarthy et al. 1992).

Analysis of variance (ANOVA) was used to examine differences within and between treatments (ration levels). Data are presented as means with their standard errors. Scheffé's multiple comparison test was used to identify the group means that were significantly different from each other at the 5% level.

Spearman rank correlation analysis was used to test the relationship between specific growth rates in the freshwater, immediate post seawater transfer, and seawater phases. Differences in food consumption rates and growth efficiencies of individual fish between the freshwater and seawater phases were tested by a paired t test.

The relationship between individual specific growth rates (SGR) and food consumption (FC) fitted a linear regression; slopes and intercepts were compared using analysis of covariance (ANCOVA). However, examination of the growth–consumption relationship after the data from the starved fish had been included showed that a power function of the form $\text{SGR} = -a + b(\text{FC})^k$ best described the relationship.

Results

Freshwater phase

At the beginning of the experiment (19 March 1993) slight differences were observed in initial mean dry weights between tanks, even though fish were randomly allocated to tanks (ANOVA, $P < 0.01$). By the end of the freshwater phase (Table 1), the mean dry weights for each of the groups were similar and not significantly different (ANOVA, $P > 0.05$). At the same time, specific growth rates (% dry body weight·day^{-1}) for the high ration groups were similar to those for the intermediate ration group but significantly higher than those for the low ration groups (ANOVA, $P < 0.05$).

Mean food consumption rates (mg dry food·g dry body weight^{-1}·day^{-1}) increased as ration size increased, up to 1% body weight·day^{-1} (ANOVA, $P < 0.001$; Table 1); significant differences existed between the two high ration groups, with

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Table 2. Effects of ration level and seawater transfer on initial and final dry weights (g), specific growth rates (SGR, % dry body weight·day^{-1}), mean consumption rates (mg dry food·g dry body weight^{-1}·day^{-1}), growth efficiencies (GE, % g dry body weight·mg dry food^{-1}), food conversion ratios (FCR, mg dry food·g wet body weight^{-1}), and condition factors (K, g·cm × 100) of individual Atlantic salmon in the immediate post seawater transfer phase (16 May – 7 June 1993).

	Rations (% body weight·day^{-1})				
	0.5	0.5	1.0	3.0	3.0
Number of fish					
Initial	36	35	40	22	39
Final	36	35	40	22	39
Final dry weight (g)	9.10±0.14*a*	9.11±0.13*a*	9.30±0.14*a*	10.45±0.28*a*	10.19±0.17*a*
SGR (%·day^{-1})	–0.38±0.04*a*	–0.45±0.05*a*	–0.26±0.04*a*	0.08±0.06*b*	–0.01±0.06*b*
Mean consumption (dry mg·g^{-1}·day^{-1})	16.52±1.29*ab*	16.51±2.01*ab*	21.86±2.24*b*	12.93±2.17*ab*	10.69±1.74*a*
GE (%)	–30.12±5.96*a*	–30.82±7.61*a*	–10.75±2.28*a*	0.89±7.87*a*	–1.63±9.11*a*
FCR	0.40±0.04*a*	0.41±0.05*a*	0.53±0.05*a*	0.33±0.05*a*	0.32±0.08*a*
K (b=2.598)	2.94±0.03*a*	2.98±0.02*a*	2.88±0.03*a*	2.89±0.05*a*	2.95±0.03*a*

Note: Values are given as the mean ± standard error. Means followed by the same letter were not significantly different at the 5% level (Scheffé's multiple comparison test). Food consumption data are taken from one sampling occasion.

Table 3. Effects of ration level and seawater transfer on initial and final dry weights (g), specific growth rates (SGR, % dry body weight·day^{-1}), mean consumption rates (mg dry food·g dry body weight^{-1}·day^{-1}), growth efficiencies (GE, % g dry body weight·mg dry food^{-1}), food conversion ratios (FCR, mg dry food·g wet body weight^{-1}), and condition factors (K, g·cm × 100) of individual Atlantic salmon in the seawater phase (13 July – 10 August 1993).

	Rations (% body weight·day^{-1})			
	0.5	0.5	1.0	3.0
Number of fish				
Initial	36	35	40	22
Final	35	35	38	21
Dry weight (g)				
Initial	13.42±0.28*a*	13.41±0.32*a*	14.46±0.34*a*	17.33±0.72*b*
Final	15.97±0.41*a*	15.66±0.48*a*	18.91±0.53*b*	29.11±1.41*c*
SGR (%·day^{-1})	0.61±0.05*a*	0.52±0.06*a*	0.93±0.07*b*	1.84±0.06*c*
Mean consumption (dry mg·g^{-1}·day^{-1})	18.75±2.10*a**	15.21±1.93*a*	21.53±2.05*a**	43.01±2.60*b*
GE (%)	36.03±5.95*a**	45.08±5.59*a*	43.0±3.67*a**	35.56±2.20*a*
FCR	0.87±0.10*a*	0.60±0.09*a*	0.62±0.06*a*	0.77±0.04*a*
K (b=3.644)	0.14±0.002*a*	0.15±0.002*a*	0.14±0.001*a*	0.15±0.002*a*

Note: Values are given as the mean ± standard error. Means followed by the same letter were not significantly different at the 5% level (Scheffé's multiple comparison test). Food consumption data are taken from one sampling occasion.

*n = 27 and 29 for ration levels of 0.5 and 1.0% body weight·day^{-1} for mean consumption, FCR, and GE.

one group having a higher rate of consumption and the other an intermediate rate when compared with the other ration groups. Growth efficiency (g dry body weight·mg of dry food^{-1}) decreased with increasing ration size, except in the aforementioned high ration group; significant differences were found to exist between the low and high ration groups (ANOVA, $P < 0.001$; Table 1).

Condition factors (K) did not differ significantly between ration levels (ANOVA, $P > 0.05$; Table 1).

Immediate post seawater transfer phase

Following 22 days in seawater there were no significant differences in dry weights between ration groups (ANOVA, $P > 0.05$; Table 2). With the exception of one of the high ration groups, specific growth rates over this period were negative; weight loss was greatest in low ration groups and decreased with increasing ration (ANOVA, $P < 0.001$).

Food consumption rates differed significantly between ration groups (ANOVA, $P < 0.001$; Table 2). The mean value in the intermediate ration group was highest and significantly different from that of one of the high ration groups ($P < 0.001$). Values in low ration groups were intermediate and did not differ significantly.

Growth efficiencies were negative and not statistically different (ANOVA, $P > 0.05$). Condition factors were not significantly different between groups (ANOVA, $P > 0.05$).

Seawater phase

Initial mean dry weights (Table 3) in the intermediate and low ration groups were similar but significantly lower than in the group fed a high ration level (ANOVA, $P < 0.001$). Final dry weights and specific growth rates increased with increasing ration level (ANOVA, $P < 0.001$).

Mean values of food consumption increased with increasing ration level (ANOVA, $P < 0.001$) but differences were only significant between the highest and lowest ration groups (Table 3). There was no significant effect of ration level on growth efficiency (ANOVA, $P > 0.05$). The condition factors

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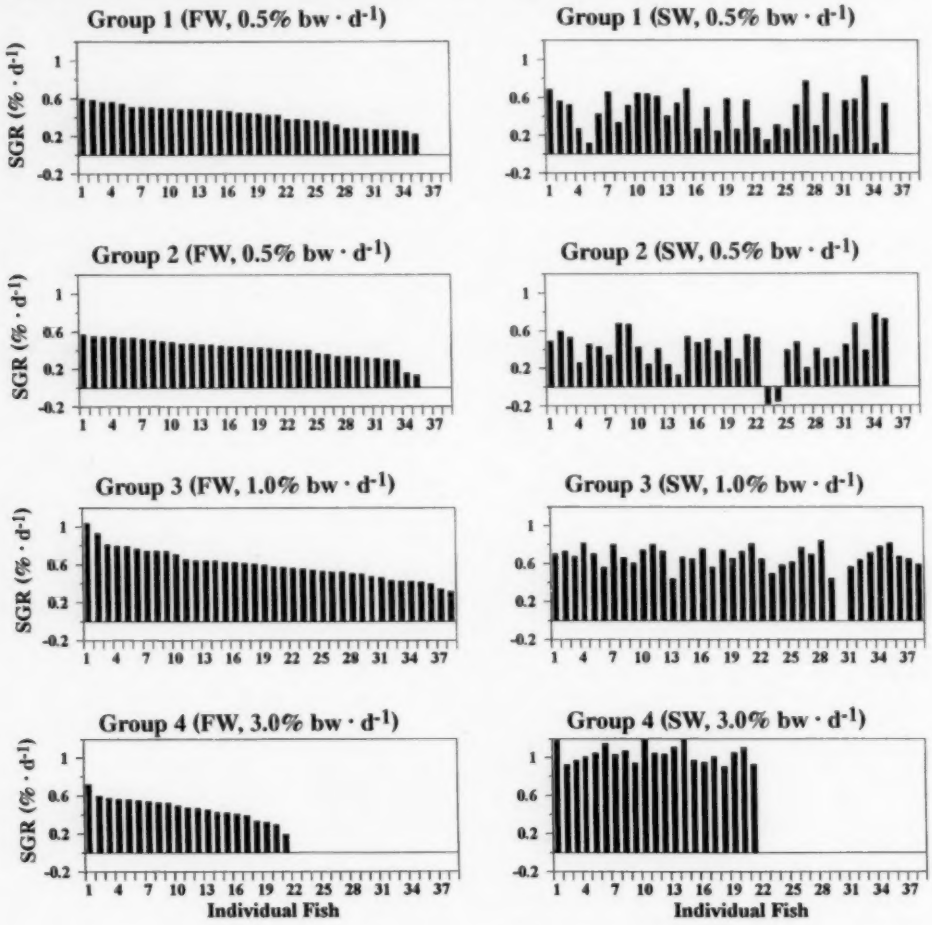
Fig. 1. Individual specific growth rates in fresh water (FW, 14 April – 15 May 1993) are ranked in descending order and the respective specific growth rates for the corresponding fish in seawater (SW, 13 July – 10 August 1993) are plotted directly opposite.


Group 1 (FW, 0.5% bw · d-1)
Group 1 (SW, 0.5% bw · d-1)
Group 2 (FW, 0.5% bw · d-1)
Group 2 (SW, 0.5% bw · d-1)
Group 3 (FW, 1.0% bw · d-1)
Group 3 (SW, 1.0% bw · d-1)
Group 4 (FW, 3.0% bw · d-1)
Group 4 (SW, 3.0% bw · d-1)
SGR (% · d-1)
1
0.6
0.2
-0.2
1 4 7 10 13 16 19 22 25 28 31 34 37
Individual Fish

did not differ significantly between ration levels (ANOVA, $P > 0.05$).

Comparisons between freshwater and seawater phases

Specific growth rates increased with increasing ration level in fresh water and in seawater. When specific growth rates of individuals for each ration group in fresh water were ranked in descending order and then compared with the specific growth rates for the corresponding fish in the seawater phase the patterns were markedly different (Fig. 1), indicating that the growth rates were not correlated ($P > 0.05$). This lack of correlation was also found when specific growth rates of individuals were compared between the freshwater and immediate post seawater transfer phases ($P > 0.05$), and between the immediate post seawater transfer and seawater phases ($P > 0.05$).

The food consumption rates observed in the immediate post seawater and seawater phases for fish on low and intermediate rations were generally similar to the rates observed for the same groups in fresh water ($P > 0.05$). Fish fed a high ration level, however, had reduced mean food consumption rates in the immediate post seawater phase (Table 2) compared with the freshwater phase (Table 1) but consumption rates in seawater (Table 3) were two times greater than rates in fresh water ($P < 0.05$).

Significant linear relationships were observed between food consumption and specific growth rates for all tanks in both fresh water and seawater when the data of the starved fish were not included (ANCOVA, $P < 0.001$). The slopes of the regression lines obtained in both phases did not differ significantly between the replicate tanks within ration levels nor between ration levels (ANCOVA, $P > 0.05$). The regression line elevations (intercepts) for groups in fresh water were similar (ANCOVA, $P > 0.05$) but the intercepts for the regression lines obtained in seawater increased with increasing ration level (ANCOVA, $P < 0.001$). Slopes and intercepts for the low ration group in fresh water were similar to values for the same tanks in seawater (ANCOVA, $P > 0.05$). The intermediate and high ration groups had steeper slopes in seawater than in fresh water (ANCOVA, $P < 0.001$).

The values for the intercepts are statistically weak because

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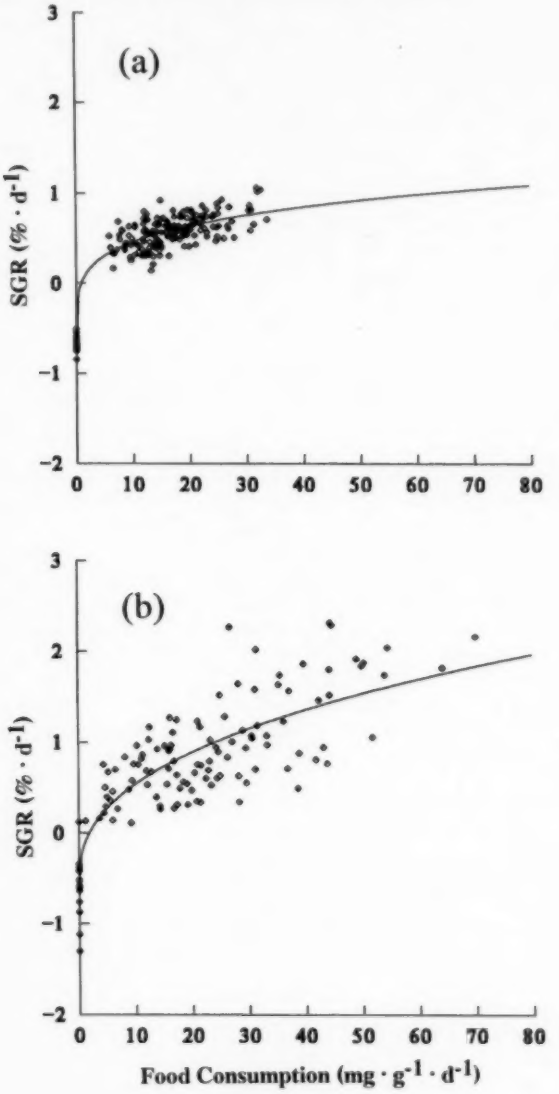
Fig. 2. Relationship between food consumption (FC, mg dry food·g dry body weight^{-1}·day^{-1}) and specific growth rate (SGR, % dry body weight·day^{-1}) in (*a*) fresh water (14 April – 15 May 1993) and (*b*) seawater (13 July – 10 August 1993), including data for starved fish. The relationships are best described by the power functions $SGR = -0.669 + 0.691(FC^{0.213})$ and $SGR = -0.552 + 0.434(FC^{0.401})$ for fresh water and seawater, respectively.



the regression lines obtained do not allow the predictions to extend beyond the range of values observed; for instance, positive specific growth rates would result at zero food consumption if the regression lines were extrapolated to the *y* axis (refer to Fig. 2). To overcome this, starved fish were included with the data to represent negative growth rates at zero food consumption. A power function best described the relationship when the data for the starved fish were included and showed that between 72 and 87% of the variation in specific growth rates was explained by variation in food consumption (Fig. 2).

Growth efficiencies for the low ration groups in seawater (Table 3) were similar to growth efficiencies observed in the freshwater phase ($P > 0.05$; Table 1). The intermediate and high ration groups showed increased growth efficiencies in seawater compared with fresh water ($P < 0.05$). The pooled data for each time interval for the length–weight relationships gave regression slopes of 2.73, 2.60, and 3.64, from fresh water to seawater, respectively. The slopes were significantly different (ANCOVA, $P > 0.05$). Condition factors were highest in the immediate post seawater transfer phase, least in seawater, and intermediate in fresh water.

Discussion

In this study, although the growth rates were highest in groups fed at the highest ration levels, growth performance in fresh water was variable and not consistently related to ration level. Differences between groups were small during this period, possibly because the mean temperature was lower in fresh water (8.8°C) than in seawater (12.9°C) and or because fish were unaccustomed to the handling associated with measurements of food consumption and growth.

During the first 22 days in seawater there was an overall decline in growth performance compared with that seen previously in fresh water; the final dry weights for all groups at the end of the period were similar to or lower than those at the beginning. This decrease in growth performance is in agreement with the findings reported by Jørgensen and Jobling (1994) for Atlantic salmon when both feeding and growth immediately after seawater transfer were suppressed. This implies that food consumption and (or) conversion efficiency had declined during this period. McCormick et al. (1989) and Shaw et al. (1975*b*) also reported negative gross food conversion efficiencies for Atlantic salmon in the immediate post seawater transfer period and the latter authors suggested that the low growth rates appeared to result from a large portion of the daily ration being required for maintenance. However, since that study estimated consumption indirectly from food offered rather than directly, as here, by X-radiography, that is, from food actually eaten, the latter conclusion must be treated with caution. Growth efficiencies have been shown to vary amongst individuals that have similar consumption rates, with some salmonid fish exhibiting higher growth rates for the same ingested ration (Carter et al. 1993*a*, 1993*b*; McCarthy et al. 1993). McCarthy et al. (1994) showed that the energetic costs of growth were higher in rainbow trout that were inefficient in protein growth for a given ration level owing to increased protein degradation and proposed that rates of protein turnover are important determinants of growth performance of individual fish. To date, it is still uncertain whether the parr smolt transformation in Atlantic salmon is associated with changes in metabolism (McCormick and Saunders 1987). Duston (1994) found that salinity had no effect on the growth rates of Atlantic salmon smolts in fresh water and at three different salinities (10, 20, and 31 ppt). Previous experience suggested that decreases in food consumption rates were more likely to result in a decline in growth performance and would not have returned to normal until approximately 20 days after seawater transfer (R. Johnstone, SOAEFD Marine Laboratory, Aberdeen,

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Scotland, unpublished data). In this investigation, feeding rates measured at this time (22 days post transfer) were similar to values observed in fresh water except in the high ration groups. Some fish had still not resumed feeding and the significantly lower mean consumption value can be attributed to inappetence in one of the high ration groups in which 33% of the fish were not feeding. Since the transfer process comprised a simple switching of the water supply to the tanks and involved no other social or physical disturbances, it is probable that the negative values observed during the immediate post transfer phase are related to disturbance of food consumption patterns resulting from the physiological challenge associated with adaptation to the hyperosmotic environment.

Growth performance of the two higher ration groups (1.0 and 3.0% body weight·day^{-1}) improved with time in seawater and may have resulted from the increased opportunity for growth occasioned by the increase in water temperatures. Usher et al. (1991) suggested that increases in daily food consumption in Atlantic salmon with time following transfer to seawater were due to increasing water temperatures. During the seawater phase (58–86 days post transfer), growth rates and growth efficiencies, which had been negative immediately after transfer, were positive and comparable to values observed in the freshwater phase, suggesting that the fish were now more fully adjusted to seawater. The highest ration group showed a two-fold increase in consumption rate during this period and a two-fold increase in mean specific growth rate over that seen in fresh water. The low ration groups had similar food consumption rates during this period, suggesting that food consumption and therefore growth were constrained by the amount of food offered. The condition factor of the fish increased in the immediate post seawater transfer period compared with values observed in fresh water, and thereafter a decline was observed; this is in agreement with the previously reported decrease in this value associated with the parr smolt transformation in Atlantic salmon (Hoar 1939; Usher et al. 1991).

Some investigators prefer to calculate food conversion ratios (FCRs) rather than growth efficiencies (GEs), and to facilitate comparisons between this and other studies investigating feeding and growth on salmonids we have included FCRs. In this investigation, fish fed a 3.0% body weight·day^{-1} ration in seawater for 86 days had a mean FCR of 0.77. Fish on the lower ration levels had mean FCRs in the range 0.60–0.87. In a study by Carter et al. (1994), Atlantic salmon fed three diets containing different levels of protein and supplementary enzymes at 2.5% body weight·day^{-1} ration levels and that were in seawater for a period comparable to the length of time used in the present study had FCRs between 0.81 and 1.13. These higher FCR values may be due to the fact that different diets were used in the two studies. Storebakken and Austreng (1987) investigated growth and FCRs in Atlantic salmon parr and found FCRs of 0.91, 1.0, and 2.66 at ratios of 0.5, 1.0, and 3.0% body weight·day^{-1}, respectively, values higher than those calculated for the same ration levels in this study. These authors, however, based their FCR calculations on the total amount of food offered to the fish and not, as here, on the amount ingested. Jørgensen and Jobling (1992) employed X-radiography to measure individual food consumption rates for Atlantic salmon parr and calculated FCRs between 0.67 and 1.04, figures comparable with those reported here.

Salinity appeared not to affect the relationship between food consumption and specific growth rates of the lowest ration groups (starved fish excluded from analysis). In contrast, the slopes for the intermediate and high ration groups during the seawater phase when fish were presumed to be acclimatized were steeper than those obtained in the freshwater phase. These findings are similar to those reported by Smith and Thorpe (1976) for rainbow trout in July to August. However, between April and May they found the reverse to be true; fish fed higher rations had lower specific growth rates in seawater than in fresh water. These authors suggested that the seasonal differences at higher rations may be due to endocrine changes associated with development. The results presented here suggest that the physiological degree of adaptation to seawater is another important albeit transitory determinant of growth. In support of this, McCormick et al. (1989) showed differing ration levels to have a significant effect on growth rate in Atlantic salmon adapted to different salinities. In contrast, other studies have shown similar growth rates between Atlantic salmon in fresh water and seawater irrespective of ration level (Shaw et al. 1975*a*) and exercise regime (Jørgensen and Jobling 1994).

These apparently disparate findings reflect the difficulty in determining the specific contribution of many interactive variables. Fish size, developmental status, feed rates, temperature, and salinity all affect consumption and growth rates. In the present study, relationships between food consumption and specific growth rates could be described by a simple linear regression model when data from starved fish were not included. However, such relationships must, in the final analysis, be treated with caution, since they suggest that positive growth rates are possible at zero consumption rates. When data from the starved fish were included a power function was found to give the best description of this relationship. Simple power functions were used to describe consumption–growth relationships in other investigations that employed X-radiography to measure individual food consumption rates, in a range of species including Atlantic salmon, Arctic char, *Salvelinus alpinus*, and carp, *Ctenopharyngodon idella* (Christiansen and Jobling 1990; Carter et al. 1992*a*, 1992*b*, 1994; Arnesen et al. 1994). In the present study, this model suggested that between 72 and 87% of the variation in individual growth rates could be explained in terms of food consumption. This is in agreement with the results of Carter et al. (1994), who found that when they used a simpler version of the model the variation in individual consumption rates explained 66–85% of the variation in growth rates of Atlantic salmon.

In this investigation freshwater growth performance was not found to be a significant indicator of subsequent seawater growth performance. This is not a surprising finding if we consider that not all salmon undergo the parr smolt transformation at exactly the same time; in a tank population, individuals will adapt to the hyperosmotic environment over different periods. Irrespective of fish size, those fish that recommence feeding quickly will be at an advantage in terms of opportunity for growth compared with those fish that start feeding later. The results reported here, which further illustrate the complexity of the interrelationships of growth rate with ration level and environment, will have important implications for farmers wishing to select individuals for increased growth under farmed conditions.

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Acknowledgements

This work was funded by a studentship award from the Aberdeen Research Consortium and by a grant from the Biotechnology and Biological Sciences Research Council. We thank the staff at the SOAEFD experimental facility at Aultbea, Wester Ross, for fish husbandry and John Webb for assistance during sampling. We especially thank Rob Fryer and Ian Wilson for their statistical advice. We also thank Neil Metcalfe and an anonymous reviewer for providing valuable comments on an earlier version of this paper.

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Mitochondrial DNA analysis of populations of lake trout (*Salvelinus namaycush*) in west-central Canada: implications for stock identification

Renato Vitic and Curtis Strobeck

Abstract: The mitochondrial DNA (mtDNA) diversity of lake trout (*Salvelinus namaycush*) distributed across west-central Canada was examined by amplified restriction fragment length polymorphism analysis (ARFLP) and DNA sequencing. A region spanning the ATPase subunit 6 and the cytochrome oxidase subunit III genes (ATPase-6-COIII) of a total of 174 fish, from 10 different populations, was surveyed for restriction site variation using a battery of eight restriction enzymes. The restriction enzymes *Hin*fI, *Sau*3A, *Alu*I, and *Hae*III resolved four mitochondrial haplotypes. The entire ATPase-6-COIII region of different ARFLP types from each population was sequenced (~1400 base pairs/individual) and a total of 17 mitochondrial haplotypes were identified. The mtDNA haplotypes identified in this study were widely distributed with common haplotypes co-occurring alongside unique haplotypes, indicating little fixed mtDNA variation between lake trout populations. Sequencing of individuals from populations in which historical transfers of fish have taken place suggests the recruitment of fish from at least one source population to a recipient population. Moreover, the co-occurrence of sequence haplotypes unique to the recipient population, alongside the introduced haplotypes, indicates the possible persistence of the original stock in the recipient population.

Résumé : On a étudié la diversité de l'ADN mitochondrial chez le touladi (*Salvelinus namaycush*) de l'ouest et du centre du Canada par analyse du polymorphisme des longueurs de fragments de restriction amplifiés (ARFLP) et séquençage d'ADN. Pour ce faire, on a recherché les variations de sites de restriction dans la région comprise entre les gènes encodant la sous-unité 6 de l'ATPase et la sous-unité III de la cytochrome-oxydase (ATPase-6-COIII) chez un total de 174 poissons provenant de 10 populations différentes en utilisant huit enzymes de restriction. Les enzymes *Hin*fI, *Sau*3A1, *Alu*I et *Hae*III ont permis de distinguer quatre haplotypes mitochondriaux. Dans chacune des populations, on a séquencé la région ATPase-6-COIII en entier chez divers types d'ARFLP (~1400 paires de bases/sujet); en tout, on a identifié 17 haplotypes mitochondriaux. Les haplotypes d'ADN mitochondrial identifiés dans la présente étude se retrouvent dans un vaste territoire; les haplotypes communs se rencontrent avec des haplotypes rares, ce qui signifie que l'ADN mitochondrial présente peu de variation fixe d'une population de touladis à l'autre. D'après les résultats du séquençage de l'ADN de sujets provenant des populations qui ont reçu des apports par le passé, il y a eu recrutement de poissons à partir d'au moins une population source en faveur d'au moins une population d'accueil. En outre, la présence simultanée d'haplotypes propres à la population d'accueil et des haplotypes introduits indique la persistance possible du stock original dans la population d'accueil. [Traduit par la Rédaction]

Introduction

Lake trout, *Salvelinus namaycush*, have a broad native distribution across most of Canada, extending from Alaska to Labrador and from the Great Lakes to several Arctic islands (Scott and Crossman 1973; Nelson and Paetz 1992). In Alberta this species is believed to be native to the Slave, Peace, Beaver, and the North and South Saskatchewan river drainages (Nelson and Paetz 1992; Mayhood 1992). Superimposed upon its native distribution is the recent transfer and introduction of lake trout from several stocks and geographic locations. During the first half of this century the introduction of fish to western Canada from as far away as the Great Lakes was not uncommon, and populations of native lake trout have undergone extensive transfer between populations within Alberta (Mayhood 1992). The consequences of these historical introductions on the genetic diversity of natural populations of lake trout are unclear, creating a need for the genetic characterization and identification of lake trout populations across their present geographic distributions.

Previous studies utilized allozyme electrophoresis for the genetic characterization of fish populations and stocks (Clayton and Ihssen 1980; Utter 1991). Although protein electrophoresis has provided valuable insights into the genetics of both natural and hatchery-propagated fish populations (Ryman and Stahl 1980; Ihssen et al. 1988), these past attempts have been "constrained because many species lack sufficient genetic divergence at polymorphic loci to permit population discrimination" (Billington and Hebert 1991). Present-day methods of directly examining genetic variation at the DNA level have greatly enhanced the resolving power and efficiency of the genetic characterization of fish populations.

Received April 11, 1995. Accepted November 21, 1995.
J12866

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Table 1. Status of 10 lake trout populations from west-central Canada.

Lake	Province	Max. depth (m)	Status of LT	Species stocked	*N*
Cold	Alta.	99.1	Native	LT, RT, CS	43
Clearwater*	Man.	—	Native	None	23
White Swan*	Sask.	—	Native	None	26
Cornwall*	Alta.	—	Native	None	8
Peerless*	Alta.	35.4	Native	None	11
Pyramid	Alta. (JNP)	19.0	Native	LT, RT, BT	9
Sassenach	Alta. (JNP)	16.0	Introduced	LT	15
Glacier	Alta. (BNP)	38.0	Native	None	15
Clearwater	Alta. (BNP)	—	Native	None	4
Minnewanka	Alta. (BNP)	99.1	Native	LT, RT, BT, CT, SPL	19

Note: *N*, sample size of fish from each population; JNP, Jasper National Park; BNP, Banff National Park; LT, lake trout; RT, rainbow trout; BT, brook trout; CT, cutthroat trout; SPL, splake; CS, coho salmon.

*Donor lakes used to stock Cold Lake.

One type of DNA that has been widely adopted for population-level genetic studies in fish is mitochondrial DNA (mtDNA). Analysis of variation in mtDNA has been successfully used to relate phylogenetic patterns to geographic distributions of fish species (Fajen and Breden 1992; Guiffra et al. 1994), the examination of evolutionary relationships among closely related taxa (Grewe et al. 1990), and the detection of hybridization and introgression (Wilson and Hebert 1993). Examinations of mtDNA diversity in populations of various fish species have indicated a few common haplotypes with a larger number of rare haplotypes that are the "mutational derivatives" of the more common types (Billington and Hebert 1991). The restriction site variation of total mtDNA among populations of lake trout has a similar pattern of genetic variation (Grewe and Hebert 1988). Recent examinations of the mtDNA variation among lake trout strains in eastern Canada suggest that such northern species exhibit some of the lowest levels of genetic divergence among fish species (Grewe and Hebert 1988; Grewe et al. 1993). Restriction analysis of total mtDNA offers a limited glimpse of the amount of genetic variation that exists between groups having low levels of genetic divergence. The development of the polymerase chain reaction (PCR) allows specific regions of DNA to be isolated and amplified, facilitating the detection of genetic differences using amplified restriction fragment polymorphism analysis (ARFLP) and DNA sequencing. Direct DNA sequencing provides the greatest resolution for the detection of genetic differences between individuals and populations and the greatest amount of information for clarifying phylogenetic relationships between organisms. For species such as lake trout, analysis of mtDNA by restriction enzymes may not be sufficient to resolve the genetic relationships at the population level and sequencing of DNA is required to adequately examine these relationships.

The present study utilized PCR, ARFLP analysis, and direct DNA sequencing to examine variation in mtDNA among populations of lake trout from west-central Canada. The sequence variation of homologous regions of lake trout mtDNA encompassing two protein coding genes, the ATPase subunit 6 (ATPase-6) gene and the cytochrome oxidase subunit III (COIII) gene, was examined using eight (four base cutters) restriction enzymes to identify different mtDNA types (haplotypes). Phylogenetic relationships between different haplotypes from different populations were then examined by sequencing this entire region of approximately 1400 base pairs (bp). The combined ARFLP data and sequence data were then used to examine the genetic structure and historical origins of populations of lake trout in west-central Canada.

Lake trout from a total of 10 populations with varying stocking histories (Table 1) were examined in this study. Included in the 10 populations examined were fish from four lakes used as donor lakes or stock sources for supplemental stocking of lake trout to Cold Lake, Alberta. The donor lakes' fish and three different age-classes, or resident subpopulations, within Cold Lake were compared to examine the stock composition of lake trout in Cold Lake and the degree of recruitment of introduced fish to the resident population. The six other resident Alberta populations of lake trout included the following: (*i*) populations that are endemic and have not received supplemental stocking, (*ii*) populations that are endemic but have received supplemental stocking, and (*iii*) one population that had been introduced. Individuals from these populations were compared with individuals from the Cold Lake donor lakes study to determine if the genetic types present in Cold Lake and the donor populations are commonly distributed across populations in Alberta.

Materials and methods

Study lakes

Where possible, information on the lakes and resident populations of lake trout was obtained from both published and unpublished sources, National Parks personnel, and stocking records. Detailed information regarding the stocking of lake trout into Cold Lake was provided by G. Sterling, Environmental Protection, Fisheries Branch, P.O. Box 275, Provincial Building, Lac La Biche, AB T0A 2C0, Canada, and McCart and Jacobson (1992).

Cold Lake, Alberta

This lake supports one of the most diverse fish faunas among Alberta lakes (Mitchel and Prepas 1990). There was an active lake trout fishery in Cold Lake in the early 1920s, but the fishery had diminished by the early 1940s and spawning surveys in the late 1980s failed to find many mature lake trout. Lake trout from several stock sources were introduced as early as 1965 to supplement the failing endemic population (McCart and Jacobson 1992). The degree to which these introduced fish have integrated with the resident fish is unclear, and the actual stock component of the resident fish in Cold Lake is unknown. Historical stocking of lake trout to Cold Lake has occurred from several source lakes: Cornwall Lake, Alberta, White Swan Lake, Saskatchewan, Clearwater Lake, Manitoba, and Lac La Ronge, Saskatchewan (see Table 2).

Clearwater Lake, Manitoba

Clearwater Lake is home to an endemic population of lake trout. No lake trout have been introduced into Clearwater Lake. It has served as a native source stock for fisheries stocking efforts both within and outside of Manitoba (G. Sterling, Environmental Protection, Fisheries Branch, P.O. Box 275, Provincial Building, Lac La Biche, AB T0A 2C0, Canada, personal communication). Fish from Clearwater Lake were introduced into Cold Lake as early as 1974 (McCart and Jacobson 1992).

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Table 2. The stocking history of Cold Lake, Alberta, 1965–1987.

Year	Month	No. stocked	Egg source	Stocking rate (no./ha)
1965	June	54 000	Lac La Ronge, Sask.	1.5
1967	Sept.	69 920	Tulip Lake, Alta.; White Swan Lake, Sask.	2.0
1968	June	121 535	White Swan Lake, Sask.	3.5
1970	Sept.	76 950	Whiteshell Lake, Man.	2.2
1971	Sept.	65 700	White Swan Lake, Sask.	1.9
1974	Aug.	91 000	Clearwater Lake, Man.; Peerless Lake, Alta.	2.6
1975	July	97 500	Clearwater Lake, Man.	2.8
1976	July	319 092	Clearwater Lake, Man.	9.1
1977	July	19 500	Clearwater Lake, Man.	Unknown
1978	July	95 040	Clearwater Lake, Man.; Cornwall Lake, Alta.	2.7
1979	June	159 355	Cornwall Lake, Alta.	4.6
1980	July	90 735	Cornwall Lake, Alta.	2.6
1981	May	208 321	Cornwall Lake, Alta.	6.0
1982	June	159 980	Cornwall Lake, Alta.	4.6
1983	July	150 000	Cornwall Lake, Alta.	4.3
1986	June	108 580	Clearwater Lake, Man.	3.1
1987	June	157 336	Cornwall Lake, Alta.	4.5
Total stocked		2 025 044		

Note: The values were modified from McCart and Jacobson (1992). The dates represent recorded stocking events and the origins of introduced fish.

White Swan Lake, Saskatchewan
This lake supports an endemic population of lake trout. No lake trout have been stocked in White Swan Lake, but fish from this lake have been introduced to smaller lakes within Saskatchewan and Alberta (Cram 1977).

Cornwall Lake, Alberta
Cornwall Lake is a small northern Alberta lake. The lake trout population is believed to be endemic to the lake, as records indicate that it has never been stocked (G. Sterling, Environmental Protection, Fisheries Branch, P.O. Box 275, Provincial Building, Lac La Biche, AB T0A 2C0, Canada, personal commuication). Because of the inaccessible northern location it is unlikely that the present lake trout population was established from unrecorded stocking efforts. It is more likely that the resident lake trout population invaded the lake from the nearby Athabasca Lake population.

Peerless Lake, Alberta
McIntyre (1990) reviewed the physical and biological characteristics of Peerless Lake. Although fish may have entered this lake from the Peace River system by adjacent Graham and Trout lakes, the population of lake trout is believed to be endemic; there is no record of stocking of lake trout.

Pyramid Lake, Jasper National Park, Alberta
Mayhood (1992) reviewed the history and status of the lake trout population of both Pyramid and Sassenach lakes. Pyramid Lake is 1 of only 10 lakes in the Rockies that are believed to have once contained a native population of lake trout. Outside of Pyramid Lake, no other native population of lake trout is believed to have existed within Jasper National Park. There are records of fish spawning and tourist catches of large lake trout from Pyramid Lake from as early as 1910, and stocking of fish prior to the turn of the century is unlikely (Ward 1974; Mayhood 1992). Although early records indicate that Pyramid Lake was not stocked prior to 1917, lake trout have subsequently been stocked at least 51 times with fish from as far away as the Great Lakes (Mayhood 1992). The native stock of fish in Pyramid Lake is likely to have undergone extensive hybridization with introduced stocks, and it is likely that the native Pyramid Lake stock of fish no longer exists.

Sassenach Lake, Jasper National Park, Alberta
The present-day population of lake trout in Sassenach Lake is considered to be the result of unrecorded introductions of fish to the lake in the early part of this century (Donald and Alger 1992; Mayhood 1992).

Clearwater Lake, Banff National Park, Alberta
Clearwater Lake supports an indigenous population of lake trout (Donald and Alger 1992). Banff National Park stocking records indicate that this lake was never stocked with lake trout (T. Hurd, Canadian Parks Service, P.O. Box 900, Banff, AB T0L 0C0, Canada, unpublished report). It is possible that the lake was invaded by fish from the nearby Glacier or Swan Lake populations. Unfortunately, the stocking records for Banff National Park are less complete than those of Jasper National Park (Mayhood 1992), and the possibility of an unrecorded introduction or invasion of Clearwater Lake cannot be dismissed.

Glacier Lake, Banff National Park, Alberta
Glacier Lake supports a native population of lake trout possibly resulting from invasion from the nearby Swan Lake (Nelson and Paetz 1992; Donald and Alger 1992). Again, the park stocking records show that this lake had never been stocked with fish of any type (T. Hurd, Canadian Parks Service, P.O. Box 900, Banff, AB T0L 0C0, Canada, unpublished report; D. Donald, Environment Canada, Environmental Conservation Branch, Room 300, Park Plaza, 2365 Albert Street, Regina, SK S4P 4K1, Canada, personal communication), but the possibility of unrecorded stocking cannot be completely ruled out.

Lake Minnewanka, Banff National Park, Alberta
This lake is one of the largest lakes in the Canadian Rockies and is subject to a high degree of recreational use. The lake supports a number of fish species, including a large native population of lake trout. A large variety of fish species have been introduced to Lake Minnewanka, with lake trout stocked as early as 1914.

Sample collection and isolation of DNA
Fish from Peerless Lake, Alberta, Cold Lake, Alberta, and the three donor lakes (Cornwall Lake, Alberta, Clearwater Lake, Manitoba, and White Swan Lake, Saskatchewan) were provided by G. Sterling, Environmental Protection, Fisheries Branch, P.O. Box 275, Provincial Building, Lac La Biche, AB T0A 2C0, Canada, and both the Saskatchewan and Manitoba Fisheries branches. Juvenile, subadult, and adult fish were separated by age. Lake trout from Clearwater Lake and Glacier Lake, Banff National Park, were supplied by D. Donald, Environment Canada, Environmental Conservation Branch, Room 300, Park Plaza, 2365 Albert Street, Regina, SK S4P 4K1, Canada. Lake trout from Lake Minnewanka, Banff National Park, Pyramid Lake, and Sassenach Lake, Jasper National Park, were supplied by the Canadian Parks Service. Fish from wild populations were collected using gill nets or collected by the commercial fisheries.

Following collection, fish were delivered to the University of Alberta DNA Repository and samples of 2–5 g of hypaxial muscle were catalogued and stored at –70°C for future research. Further tissue samples of 0.5 g were frozen in liquid nitrogen, homogenized by hand with a mortar and pestle, and resuspended in 1 mL of 0.15 M ammonium chloride, 0.01 M potassium bicarbonate, 0.1 mM disodium ethylenediaminetetraacetic acid (EDTA), (ACK) lysis buffer at 4°C. Total genomic DNA was then extracted using an Applied Biosystems Genepure 341 automated DNA extractor. Total genomic

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Table 3. Oligonucleotide primers used to amplify PCR product from lake trout.

Primer	Sequence (5′–3′)	Source
1. CST 54	GTAAATAGAGACGTATAGGAAGAG	*Oncorhynchus* spp.*
2. CST 55	GCTTCTTCGACCAATTTATGAGCC	*Oncorhynchus* spp.*
3. CST 196	CGACCAATTTATGAGCCCCACATACC	Lake trout†
4. CST 197	GAGACGTATAGGAAGAGCCATACGAC	Lake trout†
5. CST 277	CCACTATGACTAGCGACAG	Lake trout†
6. CST 278	GGTGGGCTCATGTAACGGTAAGACC	Lake trout†
7. CST 341	CCACAGGCATTATTACTC	Lake trout†
8. CST 342	GCAGCGGTGGGTTGGTTC	Lake trout†

Note: Primers 1–4 were used to amplify the entire fragment containing both of the ATPase-6-COIII genes and primers 5–8 were used to sequence the internal regions in these two genes.

*Sequences were taken from Thomas and Beckenbach (1989).

†Sequences were obtained during this study and are described in Vitic (1995).

DNA was resuspended in a Tris–EDTA buffer and stored at –20°C for subsequent genetic analysis.

Primers and polymerase chain reaction conditions

The ~1400-bp region spanning the ATPase-6-COIII genes was amplified by PCR using oligonucleotide primers designed from sequences provided by Thomas and Beckenbach (1989). Published sequences from six salmonid species were used to construct primers CST 55 and CST 54 (Table 3). This primer set proved to be unreliable for the amplification of PCR product from lake trout, and a second set of primers over the same region was constructed from sequence data obtained from lake trout (Table 3). Reaction mixtures were adjusted to optimize PCR product yields and contained 200 μM of each primer, 0.1 mM of each deoxynucleotide dATP, dTTP, dCTP, and dGTP, 1× *Thermus aquaticus* (*Taq*) magnesium-free polymerase buffer (Promega, Madison, Wis.), 0.5 U of *Taq* polymerase (Promega), and 10–500 ng of total genomic DNA, in a final volume of 100 μL.

Amplifications were performed using a Perkin-Elmer Cetus 480 DNA thermocycler under the following conditions: initial denaturation at 94°C for 4 min, annealing at 56°C for 30 s, primer extension (or synthesis) for 2 min at 72°C, followed by 40 cycles of 94°C for 15 s, 56°C for 30 s, and 72°C for 2 min, and a final cycle of 94°C for 15 s, 56°C for 30 s, and 72°C for 10 min. Products obtained following amplification were separated by gel electrophoresis on a 1% agarose gel and visualized under ultraviolet light following staining with ethidium bromide.

Amplified restriction fragment length polymorphism analysis

Amplified PCR products were digested with eight restriction enzymes: *Cfo*I, *Hae*III, *Hin*fI, *Dde*I, *Sau*3A1, *Hpa*II, *Alu*I, and *Rsa*I. Digests were performed in 20-μL volumes with 100–200 ng of amplified DNA, 3 U of enzyme, and a 1:10 dilution of the manufacturer-recommended 10× digestion buffer. Digestions were incubated overnight at 37°C, and the resulting fragments were separated by gel electrophoresis on a Bio-Rad (Richmond, Calif.) vertical gel apparatus using a 4% polyacrylamide 1× TBE (Sambrook et al. 1989) gel buffered in 0.5× TBE at approximately 300 V and 25 mA for 2–2.5 h. Following electrophoresis the restriction patterns were visualized and photographed, and the sizes of the bands were estimated by comparison with a commercially obtained 123-bp ladder (Bio-Rad). Each unique haplotype was scored on a presence or absence basis.

Sequencing

Amplified ATPase-6-COIII fragments were sequenced in 32 lake trout. Individuals were selected on the basis of their observed ARFLP types and individuals of each of the ARFLP types (A, B, C, or D) from each of the geographic locations were sequenced. A single brook trout, *Salvelinus fontinalis*, and a single bull trout, *Salvelinus confluentus*, were also sequenced as outgroups for phylogenetic analysis. Sequencing was performed directly on double-stranded PCR product. Following initial amplification, PCR products were electrophoresed on a 1% agarose gel to purify the PCR product from any unreacted primers or secondary products. Once separated, the sequence templates were excised from the agarose gel, retrieved by electroelution, and resuspended in sterile double-distilled water. Sequence reactions were obtained using the *Taq* DyeDeoxy™ terminator cycle sequencing kit (Applied Biosystems Inc., Foster City, Calif.). Sequence products were generated using the Perkin-Elmer Cetus model 9600 thermal cycler. Templates were preheated to 96°C then exposed to 25 cycles of 96°C for 15 s, 50°C for 1 s, and 60°C for 4 min; samples were then held at 4°C until they could be electrophoresed. Excess DyeDeoxy™ terminators were removed from the completed sequence reactions by purification using Centri-Sep™ spin columns (Princeton Separations Inc., Princeton, N.J.). Reaction mixtures were dried, resuspended in a mixture of 5 μL deionized formamide and 1 μL of 50 mM EDTA, pH 8.0, and separated on a 6% polyacrylamide gel using an Applied Biosystems 373A DNA sequencer.

Analysis

Following sizing, all variable sites were identified and the gene frequencies of haplotypes in each population were estimated. The probability that two alleles chosen at random from the population are different from each other, or the gene diversity of the examined loci, was calculated for all of the populations examined (Li and Graur 1991). As a test of significance the frequency of haplotypes between populations was compared using a χ^2 test of heterogeneity (Zar 1984). The ability to detect an individual type within a population is related to its frequency and the size of sample taken from that population. Thus, the discussion of the significance of the presence, absence, or frequency of a particular haplotype within a population must include some reference to the probability of detecting a haplotype within a population given a certain sample size. The probabilities of not detecting haplotypes with theoretical frequencies of 0.05, 0.10, 0.15, 0.20, and 0.25 in a population, given varying sample sizes, were calculated using the formula $(1 - P)n$, where P is the probability of detecting a particular haplotype within a population (the frequency of the particular haplotype), and n is the sample size from that population (Grewe et al. 1990).

Sequences were analyzed using the Applied Biosystems SeqEd™ DNA sequence editor. Phylogenetic analysis of sequences from all of the individuals was accomplished with phylogenetic analysis using parsimony (PAUP) of Swofford (1991). Most parsimonious trees were calculated using the heuristic search option, with random addition of trees for 10 replicates; a strict consensus tree was calculated from the shortest trees using brook trout and bull trout for rooting as outgroups. The PAUP bootstrap option with 100 replications was used to test the rigor of the topology of the strict consensus tree.

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Table 4. Four mitochondrial haplotypes identified using amplified restriction fragment analysis of the ATPase-6-COIII region of the mtDNA of lake trout and restriction fragment patterns.

(A) Haplotypes.

Haplotype	Restriction enzymes			
	*Alu*I	*Hin*fI	*Hae*III	*Sau*3AI
A	0	1	0	1
B	0	0	1	0
C	0	1	0	0
D	1	1	0	1

(B) Restriction fragment pattern sizes (bp).

*Alu*I		*Hin*fI		*Hae*III		*Sau*3AI	
0	1	0	1	0	1	0	1
687	687	786	786	528	528	868	811
277	277	408	408	270	202	506	506
225	225	246	150	192	192		57
186	165		96	180	180		
	21			110	110		
				63	64		
					63		

Note: Each restriction enzyme gave two possible patterns. In part A, composite mtDNA types were scored as presence (1) or absence (0) for each restriction pattern. In part B, restriction fragment sizes are given in base pairs.

Results

Amplified restriction fragment length polymorphism analysis

The ATPase-6-COIII regions of 174 individual lake trout were examined using the eight restriction enzymes. Some fragments were too small to be consistently visualized with ethidium bromide staining. The fragment sizes were verified by comparisons with sequence data. Of the eight restriction enzymes used, four enzymes each identified a single polymorphic site. The restriction enzymes *Hae*III, *Alu*I, *Hin*fI, and *Sau*3A1 each gave two variable restriction patterns. A 266-bp fragment in the *Hae*III restriction digest was cut, resulting in a 202-bp fragment and 64-bp fragment that was not consistently visible. Variation in the *Alu*I restriction pattern occurred when a 186-bp fragment was cut into a 165-bp fragment and a 21-bp fragment that was not consistently visible. In the *Hin*fI pattern a 246-bp fragment was cut into two smaller fragments of 150 and 96 bp. The *Sau*3A1 variant exhibited either an 868-bp fragment or an 811-bp fragment and a 57-bp fragment that was not consistently visible. No other changes in band sizes were apparent in the other bands present or other restriction patterns. Composite haplotypes were assigned on a presence (1) or absence (0) basis of each of the above two possible restriction patterns. A total of four composite mtDNA haplotypes were identified by the ARFLP digests (see Table 4), and each type differed by at least one restriction site.

A comparison of the frequency distribution of the four haplotypes in the total Cold Lake population with that in the three donor populations shows that no ARFLP haplotypes were unique to any single population and that at least one haplotype was shared between the Cold Lake and donor lake stocks (see Table 5 and Fig. 1). The apparent lack of fixed variation indicates that these four haplotypes diverged genetically (at least with respect to their mtDNA) before they were physically separated and not enough time has passed for these populations to accumulate fixed variation between the populations. Although there were no unique haplotypes, the frequency of haplotypes between Cold Lake and the donor lakes was different ($\chi^2 = 27.029$; df = 9; $p < 0.005$). The composition of haplotypes between generations in the Cold Lake stock was not significantly different ($\chi^2 = 8.105$; df = 6; $p < 0.25$); this is likely due to the small sample sizes of each group (17,

Table 5. Gene frequencies of four mitochondrial haplotypes observed among 10 populations of lake trout distributed across west-central Canada.

Lake	*N*	Haplotype	Frequency	No. of individuals/ haplotype	Gene diversity (*h*)
Cold, Alta. (combined)	43	A	0.51	22	0.61
		B	0.33	14	
		C	0.02	1	
		D	0.14	6	
Cold, Alta. (adults)	17	A	0.46	12	0.574
		B	0.46	12	
		D	0.08	2	
Cold, Alta. (subadults)	14	A	0.57	8	0.61
		B	0.14	2	
		C	0.07	1	
		D	0.21	3	
Cold, Alta. (juveniles)	12	A	0.58	7	0.571
		B	0.25	3	
		D	0.17	2	
Cornwall, Alta.	8	A	1.0	8	—
Peerless, Alta.	11	A	0.27	3	0.407
		B	0.72	8	
Clearwater, Man.	24	A	0.38	9	0.686
		B	0.29	7	
		C	0.29	7	
		D	0.04	1	
White Swan, Sask.	26	A	0.46	12	0.574
		B	0.46	12	
		C	0.08	2	
Pyramid, Alta. (JNP)	9	A	0.33	3	0.643
		B	0.22	2	
		C	0.45	4	
Sassenach, Alta. (JNP)	15	A	1.0	15	—
Clearwater, Alta. (BNP)	4	B	1.0	4	—
Glacier, Alta. (BNP)	15	A	1.0	15	—
Minnewanka, Alta. (BNP)	19	A	0.68	13	0.438
		C	0.32	6	

Note: The gene diversity (*h*) was calculated from the observed frequencies of each population (Li and Graur 1991). *N*, total sample size from each population.

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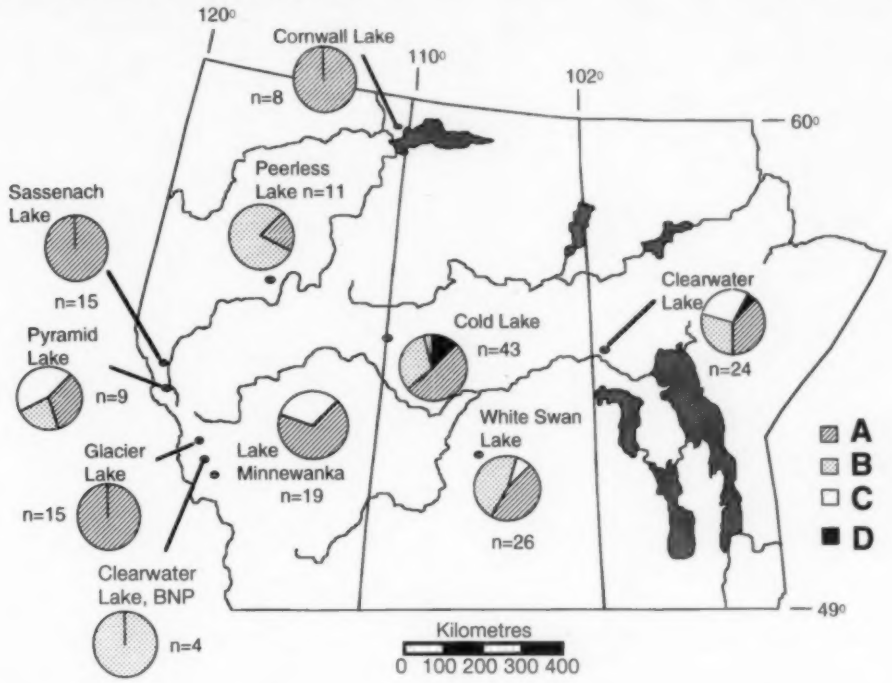
Fig. 1. The percent composition and distribution of the four ARFLP haplotypes in all 10 lake trout populations examined. Percentages and sample sizes from each population were taken from Table 5.



14, and 12). For further analysis the Cold Lake fish were grouped as one population.

Of the four haplotypes observed in Cold Lake and the three donor lakes (Cornwall Lake, Clearwater Lake, and White Swan Lake), the most common was A. It was present in all of the populations. The lowest frequency of A was observed in the Clearwater Lake population (0.38). The highest frequency was observed in the Cornwall Lake population (1.0). Only eight fish from Cornwall Lake were examined, and the failure to detect any other haplotypes may be due to the small sample size from this lake. The B haplotype was also present in two of the donor stocks and all of the Cold Lake subpopulations. The co-occurrence of two distinct mitochondrial lineages in several populations suggests that these populations cannot be distinguished phylogenetically. Clearwater Lake exhibited the greatest degree of gene diversity (0.686) and shared all four haplotypes with the Cold Lake subadult subpopulation. The C type was present in low to moderate frequencies in two of the donor lakes (0.08–0.29) but was present in low frequencies in the Cold Lake population, and was only found in the subadult population. This type may have been introduced from one of the donor lakes. The D type had the most limited distribution. It was present in only two lakes and exhibited the lowest frequencies (0.04 for Clearwater Lake and 0.14 for the total sample from Cold Lake). This suggests that the D haplotype was introduced from the Clearwater stock. Seventeen individuals were sampled from the Cold Lake adult population, and the probability of not detecting this haplotype in the adult population, given its observed frequency in the subadult population (0.07), was 29%.

The same mtDNA haplotypes observed in the Cold Lake and donor lakes, with the exception of the D type, were also observed in the six other Alberta lake populations. No new haplotypes were identified. Differences did exist in the frequencies of the different haplotypes between all of the populations examined ($\chi^2 = 101.8967$; df = 27; $p < 0.001$). The A type was present in all of the populations examined except for Clearwater Lake, but the failure to detect the A type in this lake was likely due to the small sample size (the probability of not detecting a haplotype with a frequency of 0.25 in the population was 0.422). The C type was present in Pyramid Lake and Lake Minnewanka in relatively high frequencies. The D haplotype did not occur in any other Alberta lake trout populations. Comparisons of the gene diversity within all of the populations examined indicated that Clearwater Lake, Man., had the highest level of gene diversity (0.686) followed by Pyramid Lake (0.643) and Cold Lake (0.61). The lowest levels of gene diversity were observed in Peerless Lake (0.407) and Lake Minnewanka (0.438).

Sequence analysis

Individuals of each ARFLP type (A, B, C, D) from each population were sequenced to examine the phylogenetic relationships among the four ARFLP types. The single D type from Clearwater Lake and the C type from White Swan Lake were omitted from the sequence analysis. The complete structure and sequence composition of the sequences obtained in this study are described in Vitic (1995). All of the sequence variations observed in the 32 individual lake trout sequences were single base pair substitutions with 18 variable nucleotide sites

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Table 6. Character states and position of variable nucleotide positions obtained from the ATPase-6-COIII sequence of 32 individual lake trout (sample identification (ID) lake trout no. beginning with (Sn), (P), (S), and (C), a single bull trout (Sc), and a single brook trout (Sf)).

		Site no. (bp)																	
ID	Type	261	342	354	454	532	591	618	645	919	957	995	1133	1223	1272	1280	1334	1353	1386
Sn97	B1	G	C	A	C	G	T	A	C	G	T	C	G	G	G	G	A	T	G
Sn52	B1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sn23	B1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sn151	B1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sn45	B2	—	—	C	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sn30	B3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	G	—
Sn184	B3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	G	—
Sn15	B3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	G	—
P8	B4	—	—	—	A	—	—	—	—	—	—	—	—	—	—	—	—	—	—
CB2	B5	—	—	—	—	—	—	—	—	—	—	—	—	—	A	—	—	—	—
Sn53	D1	—	—	—	—	—	—	—	—	—	—	T	—	—	—	—	—	—	—
Sn39	D1	—	—	—	—	—	—	—	—	—	—	T	—	—	—	—	—	—	—
Sn36	D1	—	—	—	—	—	—	—	—	—	—	T	—	—	—	—	—	—	—
Sn78	C1	A	—	—	—	—	—	—	A	—	—	—	A	A	—	A	—	—	—
Sn74	C1	A	—	—	—	—	—	—	A	—	—	—	A	A	—	A			
Sn70	C1	A	—	—	—	—	—	—	A	—	—	—	A	A	—	A	—	—	—
P6	C1	A	—	—	—	—	—	—	A	—	—	—	A	A	—	A	—	—	—
Sn163	C2	A	—	—	—	—	—	—	A	T	—	—	A	A	—	A	—	—	—
Sn3	A1	A	G	—	—	—	—	G	A	—	—	—	—	A	—	A	—	—	—
Sn94	A1	A	G	—	—	—	—	G	A	—	—	—	—	A	—	A	—	—	—
Sn75	A2	A	—	—	—	—	—	G	A	—	—	—	—	A	—	A	—	—	A
Sn71	A3	A	—	—	—	A	—	G	A	—	C	—	—	A	—	A	—	—	—
Sn66	A4	A	—	—	—	—	—	G	A	—	—	—	—	A	—	A	T	—	—
Sn132	A5	A	—	—	—	—	—	G	A	—	—	—	—	A	—	A	—	—	—
S5	A5	A	—	—	—	—	—	G	A	—	—	—	—	A	—	A	—	—	—
Sn4	A5	A	—	—	—	—	—	G	A	—	—	—	—	A	—	A	—	—	—
P7	A5	A	—	—	—	—	—	G	A	—	—	—	—	A	—	A	—	—	—
Sn27	A6	A	—	—	—	—	C	G	A	—	—	—	—	A	—	A	—	—	—
Sn165	A7	A	—	—	A	—	—	G	A	—	—	—	—	A	—	A	—	—	—
Sn155	A8	A	—	—	—	—	—	G	A	—	—	—	A	A	—	A	—	—	—
Sn161	A8	A	—	—	—	—	—	G	A	—	—	—	A	A	—	A	—	—	—
Sn49	A9	A	—	—	—	A	—	G	A	—	—	—	—	A	—	A	—	—	—
Sc1	Bull	—	—	—	—	—	—	G	—	—	—	A	—	A	—	A	—	—	—
Sf	Brook	A	—	—	—	—	—	—	—	—	—	—	A	—	—	—	—	—	—

Note: Individuals were taken from 10 different locations and are grouped according to their respective haplotypes.

and a total of 17 different composite haplotypes were identified (Table 6). Of the 18 variable nucleotide sites identified, 10 were informative (occurring in two or more of the individuals sequenced), and the rest consisted of single unique base pair changes. The four polymorphic restriction sites identified by ARFLP analysis were identified by sequence analysis: the *Sau*3A1 site is the result of a single A–G base change at bp 618, the *Alu*I site was the result of a single C–T change at bp 995, the *Hae*III site was the result of a single G–A change at bp 1223, and the *Hin*fI site was the result of a single G–A change at bp 1280. Six more phylogenetically informative sites were identified by sequence analysis, none of which resulted in any further restriction sites. The unique haplotypes identified by sequencing are first labelled with the restriction digest designation (i.e., belonging to the A, B, C, or D type) followed by a number denoting the haplotype identified by sequencing. The sequence analysis revealed a larger number of unique haplotypes that were not detected with the ARFLP analysis. A total of 17 types were identified by sequence analysis of 32 individuals. The number of individuals sequenced ranged from a single individual in two of the populations examined to 10 individuals from Cold Lake. The A group of mitochondrial haplotypes was the most diverse with nine haplotypes; five types were identified in the B group, two types in the C group, and a single type in the D group. A total of 10 haplotypes occurred in only one location (Fig. 2). The remaining seven types occurred in at least two populations. The A5 type was the most common type, occurring in 40% of the populations examined. The occurrence of multiple types in different geographic locations concurs with the observation from the restriction analysis.

Phylogenetic analysis of the sequence data resulted in six most parsimonious trees of differing topologies; a consensus tree was calculated from the six trees (Fig. 3). The 17 sequence haplotypes separated into four major evolutionary divisions: the B3 types; the B1, B2, B4, B5, and D1 types; the A1 types; and the A2, A3, A4, A5, A6, A7, A8, A9, C1, and C2 types. The A group of haplotypes separated into three major divisions. Two closely related types, the A3 and A9 haplotypes, branched off from all of the other A types but occurred only

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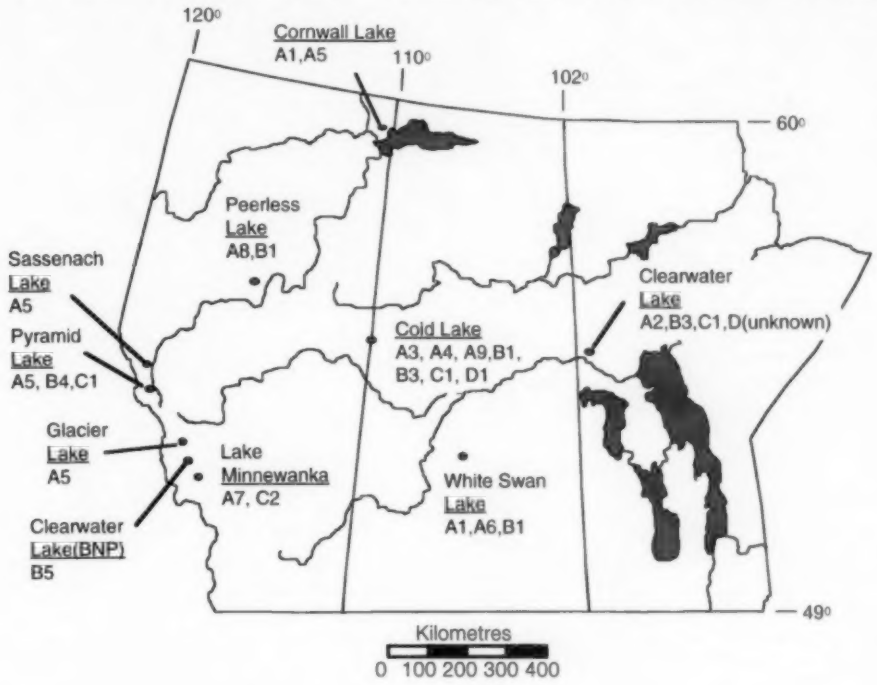
Fig. 2. Composition and distribution of haplotypes identified by sequence analysis in all 10 lake trout populations examined. The numbers of individuals sequenced from each population were as follows: White Swan Lake, Saskatchewan, $n = 4$; Clearwater Lake, Manitoba, $n = 5$; Cornwall Lake, Alberta, $n = 2$, Cold Lake, Alberta, $n = 10$; Peerless Lake, Alberta, $n = 3$; Pyramid Lake, JNP, Alberta, $n = 3$; Sassenach Lake, JNP, Alberta, $n = 1$; Clearwater Lake, BNP, Alberta, $n = 1$; Glacier Lake, BNP, Alberta, $n = 1$; Minnewanka Lake, BNP, Alberta, $n = 2$.



in the Cold Lake population. The A1 haplotype also separated out from all other A types but was shared between both White Swan Lake and Cornwall Lake. Sequence analysis of two individuals from the Peerless Lake population revealed a unique A8 haplotype that shared a single informative site with the C group of haplotypes, suggesting a recent division of the A8 type from the C group of fishes. The C group of fishes consisted of two haplotypes that were closely related to the A group. The phylogenetic relationships between the rest of the A haplotypes are unclear as the remaining A types formed a polytomy. The B haplotypes separated strongly into two groups consisting of the B3 haplotype phylogenetically separate from the remaining four B haplotypes. The distribution of the B3 type was limited to only two lakes (Cold Lake and Clearwater Lake, Man.). All of the D types consisted of a single haplotype derived from the B haplotypes.

Discussion

Restriction fragment analysis of the ATPase-6-COIII region in the mtDNA of lake trout from 10 populations resolved four mitochondrial haplotypes. Further sequence analysis revealed a total of 17 mitochondrial haplotypes. Haplotypes unique to a particular geographic location co-occurred with more commonly occurring types. Although limited by the small sample size the occurrence of several lake trout mtDNA lineages in more than one geographic location and the co-occurrence of several haplotypes unique to single locations suggest several possibilities for the origins of these populations. Firstly, these populations could have recently separated geographically and have a limited degree of genetic differentiation between them. This possibility is supported by previous examinations of northern species such as lake trout (Grewe and Hebert 1988; Billington and Hebert 1991). Mounting evidence suggests a slow rate of genetic change in the mitochondrial genomes of some fishes (Rand 1993), particularly in the salmonids (Bernatchez and Danzman 1993; Guiffra et al. 1994). Given a slow rate of genetic differentiation in the mtDNA of lake trout, the fixation of genetic differences within these populations subsequent to the most recent glacial period (20 000 – 13 000 years ago) may not have occurred.

Secondly, extensive human transfers and introductions of lake trout to geographically isolated populations may be a possible explanation for the shared haplotypes occurring in several locations. The past history of stocking practices and the possibility for the introgression of introduced types from adjacent populations certainly support this possibility. Not all possible sources of introduced lake trout (local sources such as Lac La Ronge or regional sources such as the Great Lakes) were examined in this study and fish from the populations omitted from this study may account for some of the observed variation. For example, the A5 haplotype was the most commonly distributed haplotype occurring in at least two populations that have received heavy supplemental stocking and at least one population that was established through the introduction of fish. This suggests an introduced origin for this haplotype.

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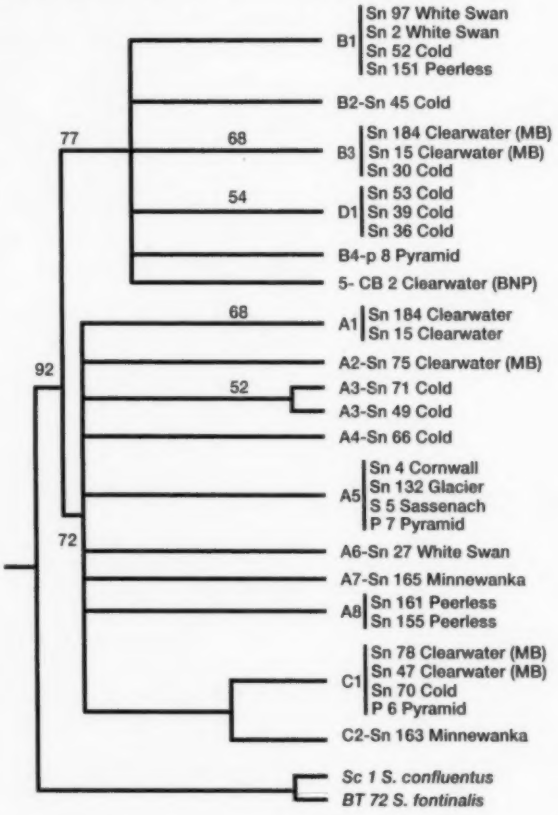
Unfortunately, we were unable to fully resolve the origin of the A5 haplotype. The A1, B1, B3, and C1 haplotypes also occurred in more than one location and may represent introduction events during the history of the resident populations of fish. Some of the highest levels of mtDNA diversity were observed in both the Cold Lake population and the Pyramid Lake population (ARFLP analysis sample sizes of 57 and 9, respectively, with 10 and 3 individuals sequenced, respectively). The higher levels of gene diversity may be the result of frequent fish introductions and the recruitment of fish to these populations.

The limited distributions of several of the sequence types, given the history of introductions of lake trout to Cold Lake from Clearwater Lake and White Swan Lake, suggest that these types have been successfully introduced to Cold Lake. For example, the Cold Lake and Clearwater Lake populations share the B3 and C1 haplotypes. The B1 type in Cold Lake was also detected in White Swan Lake, suggesting the transfer of this type to Cold Lake. The D type identified by the ARFLP analysis was detected only in the Cold Lake and Clearwater Lake populations. The successful introduction of fish from the donor lakes is also supported by the observation of an increase in the recruitment of lake trout in Cold Lake, particularly the recruitment of fish with similar morphological characteristics, such as size and color, to the more southerly populations of Clearwater Lake and White Swan Lake (G. Sterling, Fisheries Management Division, Alberta Environmental Protection, Lac La Biche, AB T0A 2C0, personal communication). Several unique types occurring in the Cold Lake population co-occur with the introduced types from the donor lakes. The A3 and A9 types, and the D1 group occur only in Cold Lake and segregate out from the other types. This suggests the possibility of the persistence of the original Cold Lake stock of lake trout. The population of lake trout in Cold Lake has experienced a decline in the recruitment of fish since about 1965 as a result of overfishing and reproductive failure owing to significant residues of DDT in the fish (Miller 1965; Paetz and Zelt 1974; Roberts 1975). The recovery of the lake trout population of Cold Lake in the 1970s failed to meet the fisheries expectations, but the recent increase in the recruitment of fish and the findings of this study suggest that the introduction of fish from at least one of the donor lakes has been successful.

The genetic characterization of populations with low levels of genetic divergence, such as those observed in this study, requires intensive sampling of the genomes to identify molecular markers useful for stock identification. The numbers of mitochondrial haplotypes identified by ARFLP analysis was consistent with the levels of diversity of lake trout strains observed in Lake Ontario using total mtDNA RFLP analysis (Grewe et al. 1993). Although direct DNA sequencing did identify a larger number of haplotypes (4 types by ARFLP and 17 types by sequencing) and similarities in the stock composition of sequence haplotypes did provide some insights into the possible origins of populations of lake trout, sequencing did not fully resolve the differences between populations. Large-scale population surveys of species such as lake trout by sequencing regions of the mtDNA over 1000 bp may not be practical because of both costs and labour intensity, but the initial sequencing of smaller numbers of individuals across the range of the species provides a basis for the further genetic screening of individuals. In our study, 32 individuals were

Fig. 3. A 50% majority bootstrap analysis of the consensus tree generated from sequence data obtained from the ATPase-6-COIII region of 32 individual lake trout, a single bull trout, and a single brook trout. The numbers on the tree branches represent the frequency of individual branch points from 100 randomly generated bootstrap replicates. Values less than 50% are omitted from the tree and these branch points are considered interchangeable. BNP, Banff National Park; MB, Manitoba.



sequenced and 18 polymorphic sites were identified. Given the base-line sequence data for these polymorphic sites presented in Vitic (1995), further screening of larger numbers of individuals is now possible using restriction enzymes for sites that correspond to polymorphic sites or allele-specific PCR for sites that do not correspond to any restriction sites (Berg et al. 1989).

In summary, restriction analysis of the mtDNA of lake trout distributed across west-central Canada revealed a broad distribution of haplotypes with little fixed variation between populations of fish. Further sequence analysis identified several unique mtDNA haplotypes co-occurring with other more common haplotypes. The co-occurrence of unique and common types and a limited amount of fixed variation between populations suggested recent geographic separation of these populations. The limited distributions of lake trout mtDNA haplotypes between Cold Lake and two lakes used as sources for supplemental stocking (Clearwater Lake and White Swan Lake) suggested successful introduction and recruitment of

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lake trout to Cold Lake. This conclusion was supported by the limited distributions of several types between these lakes and the observation of an increase in the recruitment of lake trout in Cold Lake with similar characteristics to the more southerly populations. Given the broad distribution of mtDNA types and the extensive history of human transfers of fish between populations of lake trout, we caution against the sole use of mtDNA for stock identification in lake trout as the occurrence of a single type in a particular location does not preclude its occurrence in other populations.

Acknowledgements

Thanks to David Schindler for both supervisory and financial support. The authors are grateful to John Coffin for technical support and comments on the manuscript. Thanks to G. Sterling of Alberta Environmental Protection, Fisheries Branch, Lac La Biche, Alta., and D. Donald, of Environment Canada, Environmental Conservation Branch, Regina, Sask. Our thanks to Gordon Hebert for technical assistance. This work was funded in part by Alberta Fish and Wildlife, Parks Canada, and grants from the National Science and Engineering Research Council of Canada administered under D.W. Schindler, and C.S.

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Algal productivity, algal biomass, and zooplankton biomass in a phosphorus-rich, saline lake: deviations from regression model predictions

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Abstract: Emerging evidence suggests that saline prairie lakes have relatively low chlorophyll concentrations, algal biomass, and primary production but high zooplankton biomass when compared with freshwater lakes with similar total phosphorus and total nitrogen concentrations. We investigated the accuracy of various regression models in predicting algal production, algal biomass, and zooplankton biomass in Redberry Lake, a saline (20 $g \cdot L^{-1}$), phosphorus-rich (average total phosphorus concentration 48 $\mu g \cdot L^{-1}$), moderately deep (maximum depth ca. 17 m) lake. Annual primary production rates were 7.6–8.8 times lower than rates predicted from total phosphorus models developed for freshwater lakes whereas mean summer algal biomass was 4.6–9.1 times lower than model predictions. Zooplankton biomass was 1.1–1.8 times higher than model predictions based on total phosphorus concentration, 2.0–2.8 times higher than predictions based on algal biomass, and 5.8–7.9 times higher than predictions based on chlorophyll concentration. Algal carbon turnover times (algal biomass divided by algal productivity) were short, suggesting rapid phytoplankton growth rates. Zooplankton grazing may have depressed phytoplankton accumulation rates and ultimately total primary production. Intense grazing may, in part, be a consequence of the high salinity of Redberry Lake, which limited planktivore abundance and reduced zooplankton diversity, resulting in dominance by large-bodied herbivores (*Diaptomus sicilis*, *Daphnia pulicaria*).

Résumé : De nouvelles données laissent supposer que les lacs salins des prairies renferment des concentrations relativement faibles de chlorophylle, de biomasse algale et d'éléments de production primaire, mais de fortes teneurs en biomasse de zooplancton, comparativement aux lacs d'eaux douces avec des concentrations comparables de phosphore total et d'azote total. Nous avons examiné la justesse de divers modèles de régression servant à prévoir la production algale, la biomasse algale et la biomasse de zooplancton du lac Redberry, formation saline (20 $g \cdot L^{-1}$), riche en phosphore (concentrations de phosphore total moyen, 48 $\mu g \cdot L^{-1}$) et de profondeur moyenne (prof. maximale environ 17 m). Les taux de production primaire annuels étaient de 7,6 à 8,8 fois inférieurs à ceux prévus par les modèles de phosphore total mis au point pour les lacs d'eau douce, alors que la biomasse algale moyenne était de 4,6 à 9,1 fois inférieure à celle prévue par les modèles. La biomasse de zooplancton était de 1,1 à 1,8 fois supérieure à celle prévue par les modèles si on se fonde sur le montant de phosphore total, de 2,0 à 2,8 fois supérieur à celui des prévisions sur la biomasse algale et de 5,8 à 7,9 fois supérieure à la valeur prévue basée sur la chlorophylle. Les temps de renouvellement du carbone algal (biomasse algale divisée par la productivité algale) étaient courts, ce qui laisse supposer un taux de croissance rapide du phytoplancton. Le broutage du zooplancton a peut-être réduit le taux d'accumulation de phytoplancton et, en bout de ligne, la production primaire totale. Le broutage intensif peut, en partie, être une conséquence de la forte salinité du lac Redberry, limitant l'abondance des planctophages et réduisant la diversité du zooplancton, d'où la dominance d'herbivores de grande taille (*Diaptomus sicilis*, *Daphnia pulicaria*).
[Traduit par la Rédaction]

Introduction

Saline lakes are a common feature of the Canadian prairies, particularly in the southern regions where evaporation rates exceed precipitation rates and where drainage basins frequently are closed. Total phosphorus (TP) concentrations frequently are high because phosphorus, like salinity, is readily conserved. While total nitrogen (TN) concentrations also may be high, TN/TP ratios tend to be low; ratios as low as 0.5–1.9 have been reported for saline prairie lakes in Alberta (Bierhuizen and Prepas 1985). Because chlorophyll concentrations for a given level of TP tend to decrease with a decrease in TN/TP ratio (Smith 1982), saline prairie lakes may have relatively low chlorophyll concentrations when compared with freshwater lakes with similar concentrations of TP and TN. Dissolved organic carbon (DOC) concentrations also tend to be high in saline lakes (Waiser and Robarts 1995): concentration increases with lake salinity (M.S. Evans, unpublished data; Curtis and Prepas 1993; Curtis and Adams 1995). High DOC concentrations may limit phosphorus bioavailability (Waiser and Robarts 1995), resulting in reduced primary productivity rates for a given level of TP when compared with

Received April 13, 1995. Accepted November 20, 1995.
J12869

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freshwater systems. Nevertheless, zooplankton biomass may be high relative to phytoplankton biomass when compared with freshwater lakes (Campbell and Prepas 1986). Thus, understandings developed for freshwater systems may require refining when applied to saline prairie lakes.

Regression models based on TP have been used to predict phytoplankton biomass in a wide variety of lakes (Kalff and Knoechel 1978; Watson and McCauley 1988). However, factors such as TN/TP ratios, thermal stratification, salinity, and zooplankton grazing affect TP to algal biomass relationships (Bierhuizen and Prepas 1985; Riley and Prepas 1985; Shortreed and Stockner 1986; McCauley at al. 1989) and weaken the predictive capability of TP – algal biomass models.

TP – algal biomass models can be improved by following two basic approaches. Models can be refined to include more of the variables (e.g., thermal stratification, zooplankton grazing, TN/TP ratios, salinity) that affect algal biomass. Such an approach provides for a better theoretical understanding of the factors affecting algal standing stocks. However, once these models are developed, they are more costly to utilize because of the larger number of variables that must be measured in addition to TP and algal biomass. Thus, their general usefulness may be limited. An alternative approach is to develop region-specific TP – algal biomass regression equations. While initially costly to develop, these regressions should have improved predictive capabilities.

There is substantial evidence that regression equations developed for one geographic region may be significantly different from regression equations developed for another geographic region. For example, winter oxygen depletion rates as a function of mean lake depth vary with geographic region as shown by Babin and Prepas (1985). In this study, Babin and Prepas compared their regression equation developed for 13 lakes in central Alberta with Welch et al.'s (1976) regression developed for deep, oligotrophic lakes located on the Precambrian Shield and with Barica and Mathias' (1979) regression developed for shallow, eutrophic Manitoba lakes. The regression equation was steepest for the Precambrian Shield lakes and shallowest for the Manitoba lakes, suggesting that trophic status, in addition to water column depth, was important in affecting oxygen depletion rates. Shortreed and Stockner (1986) examined the relationship between spring TP and chlorophyll concentrations for prairie, British Columbia, and Yukon lakes. They found that although TP concentrations were higher in Yukon than British Columbia lakes, chlorophyll concentrations were lower. They related low chlorophyll concentrations in Yukon lakes to the fact that zooplankton biomass was relatively high when compared with Hanson and Peter's (1984) zooplankton biomass model predictions based on TP. The large zooplankton biomass was attributed to low fish predation.

In a recent study, we investigated the accuracy of various regression models in predicting algal production, algal biomass, and zooplankton biomass in a hyposaline, eutrophic prairie lake (Evans et al. 1995). We found that annual algal production and mean summer edible algal biomass were lower than model predictions (based on TP) whereas mean summer total algal biomass and inedible algal biomass were greater than model predictions. We also determined that the TP model investigated provided an accurate predictor of zooplankton biomass whereas the algal biomass model overestimated and the chlorophyll model underestimated zooplankton biomass.

Here, we extend this research by examining the predictive capabilities of the same models used in Evans et al. (1995) for Redberry Lake, a saline, phosphorus-rich prairie lake. We were interested in determining whether or not there is a systematic trend in the ability of regression models to predict algal production, algal biomass, and zooplankton biomass in saline prairie lakes. Redberry Lake was of interest because while it has a moderately high TP concentration (48 $\mu g \cdot L^{-1}$), it has remarkably clear waters and low chlorophyll concentrations (Hammer and Haynes 1978; Robarts et al. 1992). Specifically, we investigated the following limnological predictors.

(*i*) We used Watson and McCauley's (1988) TP regression models to investigate whether or not annual primary production rates, mean summer total algal biomass, and mean summer algal composition (edible, inedible) in Redberry Lake approached values expected for freshwater lakes with similar TP concentrations.

(*ii*) We used McCauley and Kalff's (1981) algal biomass model and Hanson and Peters' (1984) TP and chlorophyll regression models to investigate whether or not zooplankton biomass in Redberry Lake was similar to that determined for freshwater lakes with similar TP and chlorophyll concentrations and algal biomass.

We investigated potential factors affecting the loss of algal biomass to better understand the possible causal factors affecting deviations in algal production and biomass from model predictions. Specifically, we investigated potential losses resulting from zooplankton grazing and from sedimentation.

Materials and methods

Study site

Redberry Lake (107°09′N, 52°43′W) is approximately 9 km wide and 10 km long. In 1974, it had a reported mean depth of 9.3 m and a maximum depth of 18 m (Hammer and Haynes 1978). Redberry Lake has decreased in area from 70 km^2 during the 1930s to 54.4 km^2 during the 1970s to its present area of ca. 45 km^2 (Van Stempvoort et al. 1993). Maximum depth is now ca. 17 m versus ca. 27 m in 1940 (Rawson and Moore 1944). Redberry Lake has increased in salinity from 14.2 $g \cdot L^{-1}$ during the 1930s to 19.4 $g \cdot L^{-1}$ during the 1970s to its present salinity of ca. 20.0 $g \cdot L^{-1}$. Decreases in lake volume and increases in salinity are believed to be due primarily to changes in land usage in the catchment basin. Interannual variations in climate also have affected the salinity and volume of Redberry Lake and other prairie, saline lakes (Hammer 1990). The major anion is sulfate (93.1%) followed by bicarbonate (3.2%), chloride (2.2%), and carbonate (1.5%); the major cations are magnesium (67.4%) and sodium (29.2%) with lesser contributions from calcium (1.8%) and potassium (1.7%).

Redberry Lake has a relatively simple crustacean zooplankton community (Rawson and Moore 1944; Moore 1952; M.S. Evans, unpublished data). The most common species are the calanoid copepod *Diaptomus sicilis* followed by the cladoceran *Daphnia pulicaria*, a seasonal dominant. *Diaptomus nevadensis*, a large-bodied copepod characteristic of saline lakes, occurs year round but in very low numbers. The cyclopoid *Acanthocyclops vernalis* is rare and seasonal in appearance. Rotifers are exceedingly rare.

Redberry Lake has a simple although modified fish community. Species reported during the late 1930s (Rawson 1946) were the ninespine stickleback (*Pungitius pungitius*), brook stickleback (*Culaea inconstans*), and fathead minnow (*Pimephales promelas*). Redberry

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Lake has now approached the upper salinity range for these species. It has been stocked with various fish species over the past 70 years in an effort to develop commercial and sport fisheries (Beak Consulting Ltd. 1989). With the exception of lake whitefish (*Coregonus clupeaformis*), these efforts failed. Some 70.5 million whitefish fry were stocked between 1940 and 1982, and a viable commercial fishery resulted from this stocking. Whitefish are unable to reproduce in Redberry Lake because of its high salinity; therefore, only a few remnants from the 1982 whitefish stocking program may remain in the lake.

Field methods

All sampling was conducted at a single 17 m deep station located near the lake center. Sampling began in 1989 when a preliminary series of samples were collected through the ice on one date in early February and one date in late March. Sampling resumed in mid-May when the lake was clear of ice: sampling was conducted biweekly until mid-October 1989 when low temperatures and poor weather precluded safe boat operation. An additional subset of data (zooplankton, chlorophyll, temperature) were obtained on a third date in July and August 1989. Monthly sampling was conducted in January, February, and March 1990 and May through October 1990.

A detailed series of collections were made during each sampling trip to determine water clarity, temperature–oxygen–pH–depth distributions, nutrient and chlorophyll concentrations, primary production, and phytoplankton abundance and composition. Phytoplankton samples were not collected in February and March 1989. Field methods are described in Robarts et al. (1992). In brief, water samples for nutrient and phytoplankton determinations were collected from 2, 8, and 16 m. Samples for chlorophyll, primary production, pH, and oxygen determinations were collected at 2-m depth intervals. Primary production was measured in situ using the ^{14}C technique with incubation periods of approximately 4 h. Percent oxygen saturation was estimated using tables developed for seawater (Green and Carritt 1967) and by assuming that the salinity of Redberry Lake was 20 $g \cdot L^{-1}$.

Duplicate zooplankton samples were collected by hauling a 50 cm diameter, 76-μm mesh net equipped with a calibrated flow meter from approximately 1 m above the bottom to the lake surface. Club soda was immediately added to the samples to relax the zooplankton. A few minutes later, zooplankton were preserved in a sugar–formalin solution.

A series of sediment traps were set in Redberry Lake, June–October 1989, to measure particulate flux rates. Trap deployment was approximately 1 month for each collection series. Duplicate traps, 10 cm internal diameter and 73 cm long, were mounted on a 75 cm wide, 60 cm high frame and set at depths of 3, 10, and 14 m. A funnel was mounted inside the bottom of the trap and over a 500-mL plastic bottle to which mercuric chloride had been added as a preservative. Samples first were sieved to remove zooplankton and then were freeze dried. Following that, subsamples were analyzed for organic carbon.

Laboratory methods

Laboratory methods for nutrient analyses and primary production are described in Robarts et al. (1992). Phytoplankton and crustacean zooplankton were identified to the lowest practical taxonomic level. Enumeration methods have been described in detail in Evans et al. (1995).

For phytoplankton biomass determinations, at least 20 individuals of a given phytoplankton species were measured for length and width determinations. Cell biovolume was then estimated from previously derived formulae for common phytoplankton shapes (Earle et al. 1987). Phytoplankton abundances were expressed as cell numbers (cells per litre) and wet weight biomass (milligrams per litre). All cyanobacteria and dinoflagellates, gelatinous green algae, and algae that were longer than 40 μm (as a colony or solitary cell) were assumed to be inedible.

We also estimated the percent chlorophyll content of the phytoplankton assemblage for each sampling date and depth stratum. We estimated this by dividing mean chlorophyll concentration by mean algal biomass and then multiplying the result by 100. We were particularly interested in determining whether or not percent chlorophyll content of algal biomass (wet weight) approached the 1% value suggested by Ahlgren (1970) and generally found appropriate by McCauley and Kalff (1981).

We also estimated the turnover time of phytoplankton biomass owing to primary production. This enabled us to identify periods in which algae were rapidly growing and periods in which growth rates were low. For each sampling date, turnover time owing to primary production was estimated by dividing total areal algal biomass (grams carbon per square metre) by the areal primary production estimate for that day. Phytoplankton wet weight biomass was converted to areal carbon by using a 0.13 conversion factor (Strickland 1960) and based on a 16-m sampling depth of the water column. Turnover times were estimated for the upper and lower strata (see below) and for the entire water column.

Zooplankton abundances were converted to dry weight on the basis of length determinations of the various zooplankton taxa and developmental stages within a given taxon. Length – dry weight regression calculations (Evans et al. 1995) were then used to convert abundances (numbers per cubic metre) to dry weight biomass (milligrams per cubic metre). A conversion factor of 10.0 was used to convert zooplankton dry weight to wet weight.

We estimated zooplankton filtering rates to investigate the potential impact of zooplankton grazing on phytoplankton biomass and composition (i.e., percent edible versus inedible algae). The regression developed by Knoechel and Holtby (1986) for cladocerans fed on *Pandorina* was used for *Daphnia pulicaria*. A mean filtering rate of 0.3 $mL \cdot animal^{-1} \cdot day^{-1}$ was used for nauplii, 1 $mL \cdot animal^{-1} \cdot day^{-1}$ for cyclopoid copepods, and 2 $mL \cdot animal^{-1} \cdot day^{-1}$ for *Diaptomus sicilis* and *Diaptomus nevadensis* (McNaught et al. 1980; Wetzel 1983).

The water column was divided into two depth strata: the upper layer, which extended from the surface to 10 m, and the deep layer, which extended from 11 to 16 m. For each variable of interest, mean values for a particular sampling time were calculated for the upper and deep layers. Water column averages also were calculated for use in the regression models investigated.

Comparisons with existing regressions

We used Watson and McCauley's (1988) TP regression models (eqs. 1, 2, 4, and 5) to investigate whether or not annual primary production rates, mean summer total algal biomass, and mean summer algal composition (edible, inedible) in Redberry Lake approached values expected for freshwater lakes with similar TP concentrations. The annual production models are based on mean TP concentration during the ice-free season and volumetric production in the euphotic zone. The algal biomass models are based on mean summer TP concentration and algal biomass for the entire water column.

We investigated three predictors of zooplankton biomass in Redberry Lake. All models were based on mean biomass over the ice-free season. We used Hanson and Peters' (1984) model based on mean TP concentrations (eq. 1) and mean chlorophyll concentrations (eq. 2) during the ice-free season. Zooplankton biomass was expressed as dry weight. We used McCauley and Kalff's (1981) phytoplankton model (eq. 3) for based on mean algal biomass for the ice-free season. Zooplankton biomass was expressed as wet weight.

Results

General limnological features of Redberry Lake

Redberry Lake is sufficiently deep to thermally stratify

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Table 1. Mean upper and lower water column physicochemical characteristics in Redberry Lake, for the 1989 and 1990 ice-free period.

	1989		1990	
	Upper	Lower	Upper	Lower
Secchi disc (m)	7.3	—	4.7	—
	(3.0–9.8)		(2.8–5.8)	
Temperature (°C)	14.1	3.6	11.6	4.5
	(1.4–24.0)	(0.4–7.2)	(1.1–20.2)	(0.7–12.6)
pH	9.0	8.9	8.9	8.8
	(8.7–9.2)	(8.7–9.2)	(8.6–9.5)	(8.6–9.3)
Oxygen ($mg \cdot L^{-1}$)	8.4	9.1	8.9	10.2
	(7.0–10.2)	(4.9–10.8)	(6.9–11.2)	(8.1–10.9)
Percent saturation	89.1	81.6	91.7	89.9
	(74.1–97.2)	(69.6–95.0)	(84.5–94.8)	(77.7–99.6)

Note: Ranges are shown in parentheses. The upper water column is 0–10 m and the lower is 11–16 m.

Table 2. Mean upper and lower water column nutrient concentrations in Redberry Lake for the 1989 and 1990 ice-free period.

	1989		1990	
	Upper	Lower	Upper	Lower
Total phosphorus	42.8	54.1	45.4	50.7
	(38–70)	(37–120)	(38–54)	(47–54)
Dissolved phosphorus	34.5	43.1	36.4	40.9
	(27–70)	(38–80)	(30–42)	(30–47)
Soluble reactive phosphorus	20.3	24.6	17.6	19.4
	(13–30)	(15–33)	(11–28)	(14–28)
Particulate nitrogen	26.4	43.8	39.6	62.4
	(<10–34)	(<10–94)	(<10–103)	(12–154)
Ammonium	18.7	23.2	24.4	23.0
	(14–29)	(14–57)	(20–36)	(19–27)
Nitrite–nitrate	<2	<2	<2	<2
Particulate organic carbon	410	471	340	491
	(131–772)	(316–881)	(15–996)	(173–952)
Dissolved organic carbon ($mg \cdot L^{-1}$)	34.5	36.7	34.5	36.3
	(33.5–36.8)	(34.7–38.3)	(31.6–37.5)	(35.8–36.7)
Silicon ($mg \cdot L^{-1}$)	0.25	0.40	0.25	0.55
	(0.07–0.54)	(0.19–0.61)	(0.02–0.72)	(0.16–0.72)

Note: Ranges are shown in parentheses. The upper water column is 0–10 m and the lower is 11–16 m. Concentrations are in micrograms per litre except as noted.

(Table 1, Fig. 1). Thermal stratification developed within 2 or 3 weeks after ice-out and persisted until October. The water column remained well oxygenated during this period. Water clarity generally was high with Secchi disc depth averaging 7.3 m for the 1989 and 4.5 m for the 1990 ice-free season. Water clarity was lower in July–October 1990 than 1989.

TP concentrations were moderately high (Table 2, Fig. 1) and at levels characteristic of mesotrophic systems (Wetzel 1983). Ammonium occurred in similar concentrations as soluble reactive phosphorus while nitrite–nitrate generally was below detection limits ($2\ \mu g \cdot L^{-1}$). Silicon concentrations also tended to be low. Phosphorus, nitrogen, organic carbon, and silicon concentrations were only slightly higher in the deep layer than in the upper layer. Overall, there was no evidence of a significant accumulation of dissolved nutrients in the deep layer through summer.

Primary productivity rates (Figs. 2*a* and 2*b*) exhibited strong seasonal variation, with most of the production occurring in the upper layer. Nevertheless, ca. 19% of annual production occurred in the deep layer. Productivity rates peaked in midsummer in 1989 and late summer in 1990. Algal-carbon turnover times (Figs. 2*c* and 2*d*) were rapid in the warm upper layer (mean 2.2 days) but substantially longer in the cold deeper layer (mean 13.3 days). Water column averages for the ice-free season were 2.9 days in 1989, 6.1 days in 1990, and 4.1 days for the 2 years of study.

Chlorophyll concentrations and algal biomass (Fig. 3) in the surface layer tended to follow a bimodal pattern in 1989 with a spring pulse immediately following ice-out, a crash by early June, and then increasing concentrations in late summer. A similar pattern was observed in 1990, although there was no autumn increase in algal standing stocks. The chlorophyll content of algal cells varied seasonally, tending to be higher in spring and autumn than midsummer and in the deep than the

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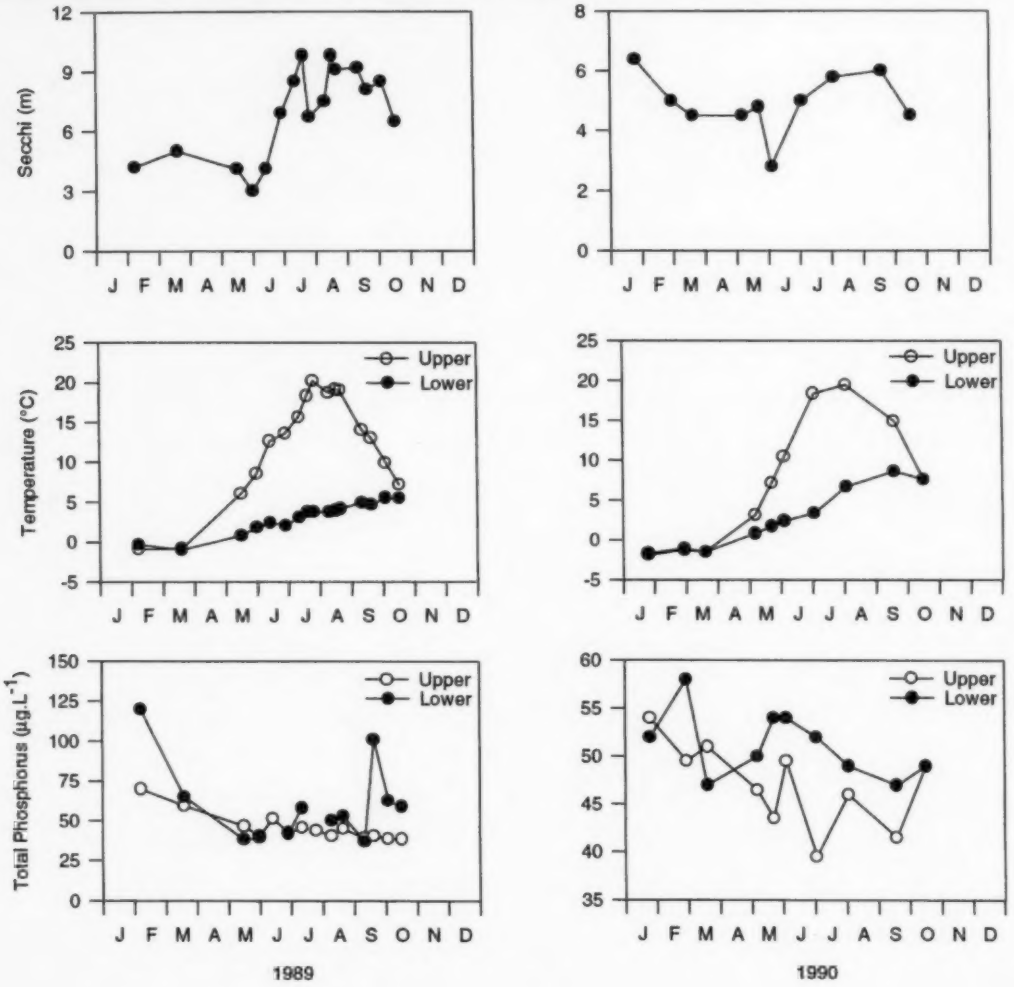
Fig. 1. Secchi disc depth, mean water temperature, and total phosphorus concentration in Redberry Lake during 1989 and 1990. Mean water temperature and total phosphorus concentration are shown for the upper and deep layers.



shallow layer (Figs. 3*e* and 3*f*). Percent chlorophyll of algal biomass ranged from 0.10 to 0.69% and averaged 0.40%. Inedible algae tended to be more abundant than edible algae (Fig. 4), especially in the upper layer.

Total zooplankton biomass (Figs. 5*a* and 5*b*) was relatively constant in 1989 with little seasonal variation; seasonal variation was more pronounced in 1990. *Diaptomus sicilis* and *Daphnia pulicaria* were the primary contributors to total biomass. *Daphnia pulicaria* appeared later in the zooplankton assemblage in 1990 than in 1989 and persisted for a shorter time. Zooplankton biomass was relatively low in May 1990 when compared with that in May 1989. The low zooplankton biomass may be related to the colder 1989–1990 winter and lower 1990 spring chlorophyll concentrations than during the 1st year of study.

Estimated zooplankton filtering rates (Figs. 5*c* and 5*d*) were relatively constant throughout 1989. *Daphnia pulicaria* was the predominant filter feeder during summer–autumn 1989 and late summer – early autumn 1990, while *Diaptomus sicilis* was the dominant one during the remainder of the year. During most of the 1989 ice-free season, estimated zooplankton filtering rates ranged from 100 to 300 $mL \cdot L^{-1} \cdot day^{-1}$. In 1990, rates ranged from 12 to 670 $mL \cdot L^{-1} \cdot day^{-1}$ with the lowest rates in May following a relatively cold winter. The inverse of this rate is the turnover time of the water column owing to zooplankton filtering (Figs. 5*e* and 5*f*). Times were 3–10 days in 1989 and averaged 6.8 days. Excluding the first sampling date in May 1990 when zooplankton biomass was very low, times ranged from 1.5 to 18.8 days and averaged 8.1 days.

Particulate flux rates, which were determined only in 1989, varied seasonally (Table 3). Mean dry weight flux was highest in late spring – early summer and in early autumn. Flux rates were similar in surface and deep traps, suggesting that most of the flux originated from the upper, productive layer. Total flux

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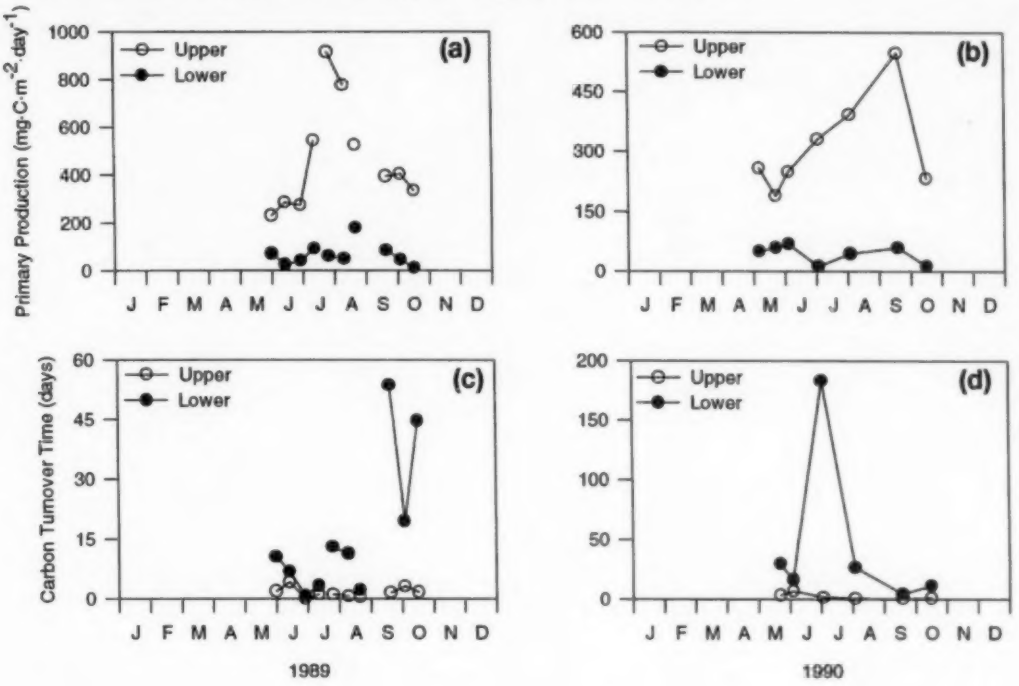
Fig. 2. Daily primary productivity rates in Redberry Lake during (*a*) 1989 and (*b*) 1990 for the upper and deep layer. Also shown are carbon turnover times (*c* and *d*) owing to algal production in the upper and deep layers in 1989 and 1990.



for the June 16 – October 17 period was 129.8 g·m^{-2}. Organic carbon flux rates ranged from 0.08 to 0.17 g C·m^{-2}·day^{-1}. The total flux of particulate organic carbon from June 16 to October 17 was 12.1 g C·m^{-2}. Assuming that flux rates were similar in May–June to those in September–October, total organic carbon flux for the ice-free season was 15.4 g C·m^{-2}.

Comparisons with regression models

Primary production, algal biomass, and algal composition

Annual primary production in Redberry Lake was from 7.6 (1990) to 8.8 (1989) times lower than Watson and McCauley's (1988) regression model predictions based on TP (Table 4). Similarly, mean summer algal biomass was 4.6–9.1 times lower than model predictions. Edible algal biomass was 2.3–7.9 times less abundant whereas inedible alga biomass was 6.7–9.6 times less abundant than model predictions. Concentrations were below the lower 95% confidence intervals established by Watson and McCauley (1988) for their TP – algal biomass regressions (Fig. 6). The proportion of edible and inedible algae was close to model predictions.

Predictors of zooplankton biomass

Mean zooplankton biomass in Redberry Lake was slightly greater (a factor of 1.1 in 1989 and a factor of 1.8 in 1990) than Hanson and Peters' (1984) model predictions based on TP and 2.0–2.8 times greater than McCauley and Kalff's (1981) model predictions based on algal biomass (Table 5). Hanson and Peters' (1984) chlorophyll model was the poorest predictor of biomass, with zooplankton biomass 5.8–9.6 times greater than model predictions.

Discussion

Predictors of algal biomass and production

Previous research conducted in Alberta saline lakes has suggested that saline prairie lakes have relatively low chlorophyll concentrations and productivity when compared with freshwater lakes with similar concentrations of TP (Bierhuizen and Prepas 1985; Campbell and Prepas 1986). Redberry Lake also appears to follow that trend. Total annual primary production in the euphotic zone was 7.6–8.8 times lower than Watson and McCauley's (1988) TP-based model predictions, while total summer algal standing stocks were 4.6–9.1 times lower than model predictions. The relatively low algal biomass and production in Redberry Lake could be due to several factors: thermal stratification (Riley and Prepas 1985), low TN/TP ratios (Bierhuizen and Prepas 1985), or some other aspect of salinity (Campbell and Prepas 1986).

Thermal stratification

Redberry Lake is sufficiently deep to thermally stratify during the ice-free season. Thermal stratification permits the rapid loss of phosphorus (through the settling of particles) from the water column. The thermocline also can serve as a barrier to the vertical exchange of nutrients, which are regenerated in the hypolimnion and at the sediment–water interface. Moreover, because of the lower deep-water temperatures in stratified than

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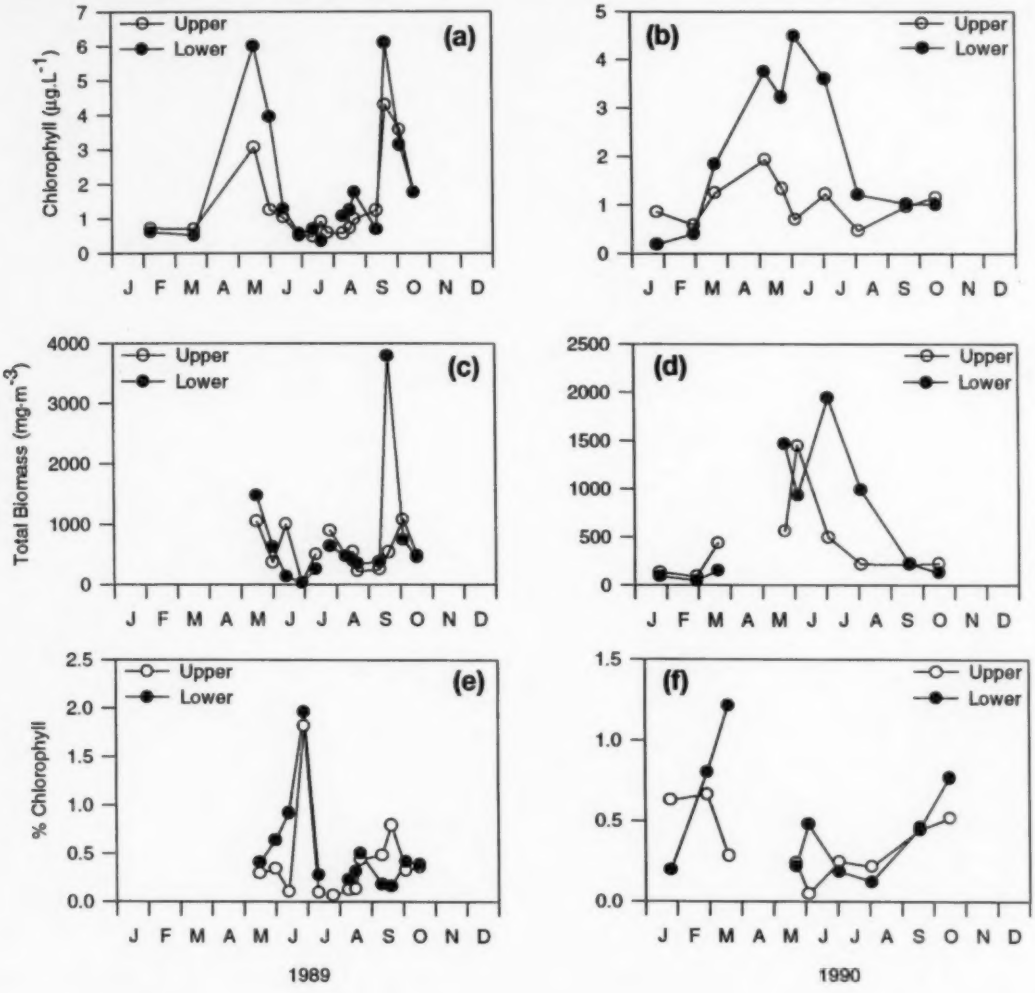
Fig. 3. Chlorophyll concentration in Redberry Lake during (*a*) 1989 and (*b*) 1990 for the upper and deep layer. Also shown are algal wet weight biomass (*c* and *d*) and percent chlorophyll content of biomass (*e* and *f*) in the upper and deep layers in 1989 and 1990.



well-mixed lakes, nutrient regeneration rates will be slower in stratified lakes than well-mixed lakes with similar dissolved oxygen concentrations. These factors may account for the fact that summer chlorophyll concentrations tend to be higher in well-mixed lakes than thermally stratified lakes with similar concentrations of TP in spring (Riley and Prepas 1985).

The physical barrier created by the thermocline, however, does not appear to be the primary factor limiting algal productivity and standing stocks in Redberry Lake. Deep-water phosphorus, nitrogen, and silicon concentrations were similar to shallow water concentrations. Nor does thermal stratification, per se, seem important. We used Riley and Prepas' (1985) regression model, which predicts mean summer chlorophyll concentrations for stratified lakes on the basis of mean spring TP concentrations. With a mean spring TP concentration of 50 $\mu g \cdot L^{-1}$, Redberry Lake had a predicted mean summer chlorophyll concentration of 14.8 $\mu g \cdot L^{-1}$ versus an actual mean value of 1.6 $\mu g \cdot L^{-1}$ in summer 1989 and 0.7 $\mu g \cdot L^{-1}$ in summer 1990.

Sedimentation

Sedimentation does not appear to be a significant lost term for algal production from the surface, productive layer of Redberry Lake. We estimated that organic carbon flux rates averaged 98.2 mg $C \cdot m^{-2} \cdot day^{-1}$ or 15.4 g $C \cdot m^{-2}$ for the 1989 ice-free season. This compares with an estimated annual production rate of 93.7 g $C \cdot m^{-2} \cdot year^{-1}$ (Robarts et al. 1992) with 80% of this production or 75.0 g $C \cdot m^{-2} \cdot year^{-1}$ occurring in the upper layer. Thus, only about 22% of the upper layer primary production in 1989 may have been exported to the deep layer through sedimentation.

Salinity

High-salinity lakes may have low TN/TP ratios (Bierhuizen

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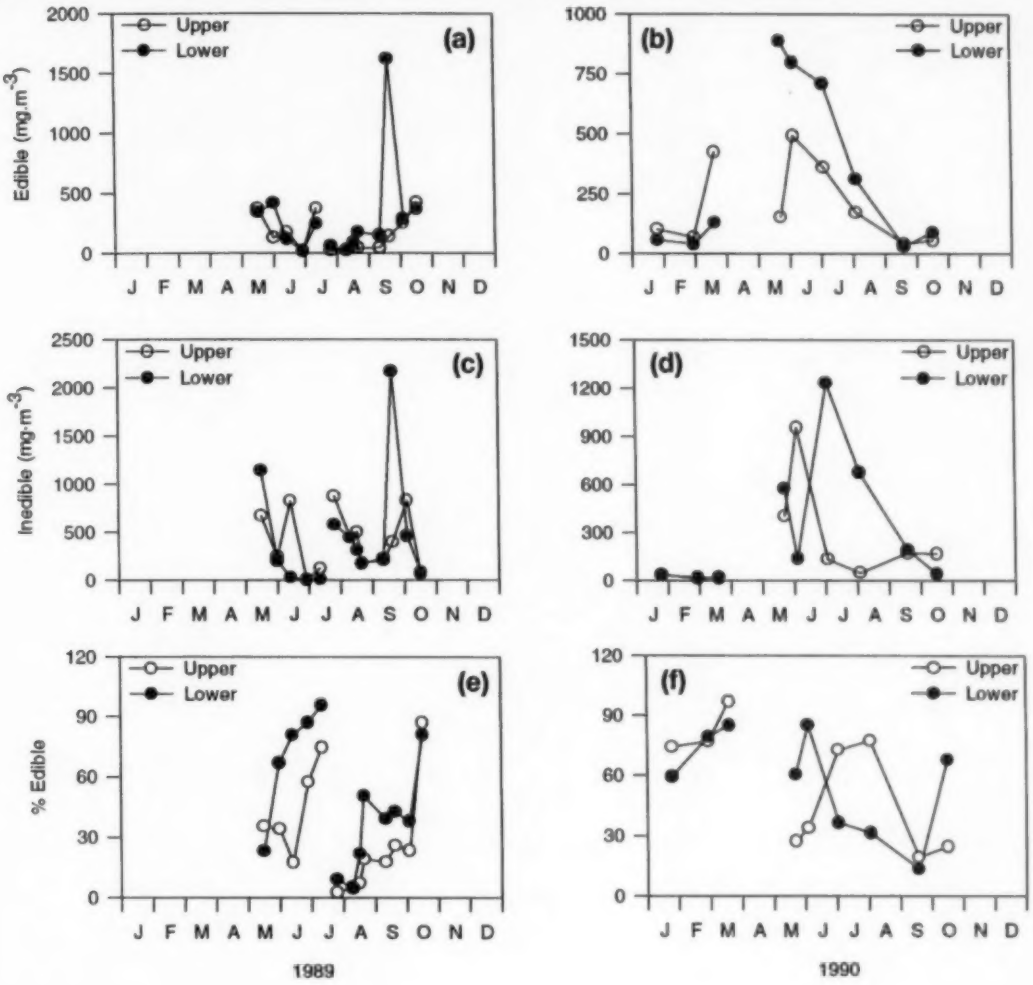
Fig. 4. Edible algal biomass (wet weight) in Redberry Lake during (*a*) 1989 and (*b*) 1990 for the upper and deep layer. Also shown are inedible algal biomass (*c* and *d*) and percent edible algae (*e* and *f*) in the upper and deep layers in 1989 and 1990.



and Prepas 1985) that can, in turn, result in low chlorophyll concentrations for a given concentration of TP (Smith 1982). To investigate the effect of the TN/TP ratio on algal standing stocks in Redberry Lake, we compared mean summer chlorophyll concentrations with values predicted from Smith's (1982) TP and TN regression model. However, because we had not measured total dissolved nitrogen concentrations, we do not have a precise estimate of the TN/TP ratio during our study period. Thus, we generated an upper and lower estimate for this ratio. A lower estimate, in which TN is based on the mean sum of particulate nitrogen, ammonia, and nitrite–nitrate concentrations for the ice-free season, is 1.1–1.7. An upper estimate, based on a total dissolved nitrogen measurement in February 1992, is 9.1. Using these lower and upper TN/TP estimates, Smith's model predicts that mean summer chlorophyll concentrations in Redberry Lake were 3.3–10.5 $\mu g \cdot L^{-1}$ versus actual values of 0.7–1.6 $\mu g \cdot L^{-1}$. While closer than estimates based on Riley and Prepas' (1985) thermal stratification model, actual values are still 2–15 times lower than predicted values.

Campbell and Prepas (1986) also determined that Smith's models underestimated chlorophyll concentrations in the three shallow (maximum depth 1.7–3.1 m), saline (9400 – 55 000 $\mu S \cdot cm^{-1}$) lakes that they investigated. The degree to which Smith's model underestimated chlorophyll concentration was larger than in our study, ranging from 70- to 625-fold. Their study lakes also differed from ours in three important aspects. First, TP concentrations were higher, ranging from 2310 to 13 304 $\mu g \cdot L^{-1}$, or 45–260 times higher than in Redberry Lake. Second, TN concentrations were higher, ranging from 3923 to 11 517 $\mu g \cdot L^{-1}$, while TN/TP ratios ranged from 0.9 to 1.7. Third, sodium was the dominant cation in their study lakes whereas magnesium predominates in Redberry Lake. The reasons why these Alberta lakes had such high TP and TN concentrations are unknown; apparently, some aspect of salinity allows TP and TN to accumulate to very high levels, exceeding

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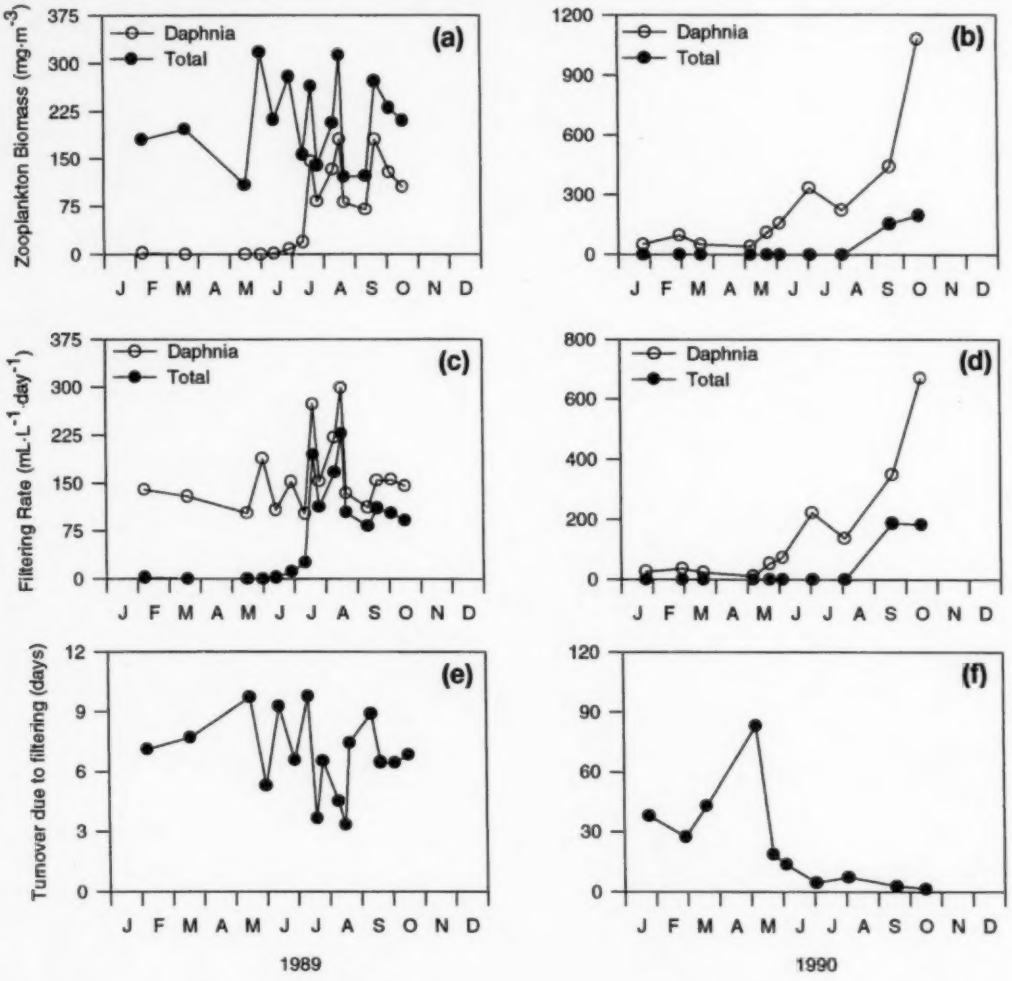
Fig. 5. Zooplankton dry weight biomass in Redberry Lake during (*a*) 1989 and (*b*) 1990. Also shown are estimated zooplankton filtering rates (*c* and *d*) and turnover times owing to grazing (*e* and *f*) in 1989 and 1990.



those observed in Redberry Lake. Moreover, the availability of these nutrients appears limited, and as a consequence, algal production rates are low (Campbell and Prepas 1986). Similarly, Waiser and Robarts (1995) determined that only a small fraction of the soluble reactive phosphorus in Redberry Lake was available to the algal community.

Zooplankton grazing

Another factor potentially affecting algal standing stocks and production is zooplankton grazing. Intense zooplankton grazing pressure may severely depress algal standing stocks and, ultimately, total phytoplankton production rates. There is some evidence that intense grazing pressure from the zooplankton community was an important factor affecting the low standing stocks of algae in Redberry Lake. We estimated that zooplankton theoretically were capable of clearing the water column of edible algae in 6.8 days in 1989 and 8.1 days in 1990. We also estimated that the algal carbon turnover time owing to primary production was 4.1 days in 1989 and 8.1 days in 1990. These algal biomass turnover times owing to primary production are within a factor of 1.0–1.7 times the turnover rates owing to filtering. This suggests that much of the loss of algal biomass could have been due to grazing.

Predictors of zooplankton biomass

TP was a relatively accurate predictor of zooplankton biomass in Redberry Lake. This was somewhat unexpected because TP overestimated algal production in Redberry Lake. Similarly, TP was a relatively accurate predictor of zooplankton biomass in Humboldt Lake, although as in Redberry Lake, TP underestimated algal production. Zooplankton biomass was some 1.1–1.8 times higher than model predictions in Redberry Lake and 0.9–1.3 times higher than model predictions in Humboldt Lake. We also applied Hanson and Peters' (1984) TP regression model to Campbell and Prepas' (1986) study lakes. We found that, in comparison with predictions based on chlorophyll and

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Table 3. Particulate flux rates ($g \cdot m^{-2} \cdot day^{-1}$, dry weight) in Redberry Lake, 1989.

	3 m	10 m	15 m
Total flux			
June 16 – July 12	1.40	1.47	1.18
July 12 – August 10	0.71	0.84	0.70
August 10 – September 11	0.71	1.03	1.10
September 11 – October 17	1.14	1.23	1.15
Organic carbon			
June 16 – July 12	0.14	0.07	0.08
July 12 – August 10	0.08	0.08	0.10
August 10 – September 11	0.10	0.10	0.09
September 11 – October 17	0.08	0.12	0.17

Table 4. Comparison of predicted versus actual summer inedible and edible algal biomass ($\mu g \cdot L^{-1}$ wet weight) and annual production ($g\ C \cdot m^{-3} \cdot year^{-1}$) in the euphotic zone for Redberry Lake according to Watson and McCauley's (1988) model.

	Predicted	Actual	Ratio
Total annual productivity			
1989	51.1	5.8	8.8
1990	51.5	6.8	7.6
Inedible algae			
1989	2992	312	9.6
1990	3017	449	6.7
Edible algae			
1989	923	117	7.9
1990	926	409	2.3
Total algae			
1989	3915	429	9.1
1990	3942	858	4.6

Note: Mean summer TP concentration was 46.9 $\mu g \cdot L^{-1}$ in 1989 and 47.2 $\mu g \cdot L^{-1}$ in 1990. Ratios are predicted/actual values.

algal biomass (see below), TP was a relatively accurate predictor of zooplankton biomass. Hanson and Peters' TP model predicts a zooplankton wet weight biomass of 6410 $mg \cdot m^{-3}$ for Fluevog, 9725 $mg \cdot m^{-3}$ for Peninsula Lake, and 31 365 $mg \cdot m^{-3}$ for Oliva versus actual values of 13 000, 14 000, and 82 000 $mg \cdot m^{-3}$, respectively. Thus, zooplankton biomass was some 1.4–2.6 times higher than model predictions based on TP.

Algal biomass was a less accurate predictor than TP of zooplankton biomass in Redberry Lake, with zooplankton standing stocks approximately 2–3 times higher than model predictions. However, zooplankton biomass was within the 95% confidence intervals established by Campbell and Prepas (1986) for McCauley and Kalff's (1981) algal biomass regression. We also reexamined Campbell and Prepas' (1986) data for their three saline lakes to determine whether or not McCauley and Kalff's algal biomass model overestimated zooplankton biomass.

As we noted in Evans et al. (1995), Campbell and Prepas (1986) estimated phytoplankton biomass on the basis of chlorophyll and the assumption that chlorophyll accounted for 1% of algal biomass. However, phytoplankton inhabiting saline, low TN/TP lakes may have a lower chlorophyll content than the 1% value found to be typical for freshwater lakes. We

Fig. 6. Mean summer inedible and edible biomass in Redberry Lake in 1989 and 1990 versus predicted values from Watson and McCauley (1988). Redrawn from Watson and McCauley (1988) showing model predictions and 95% confidence intervals. See text for further explanation.

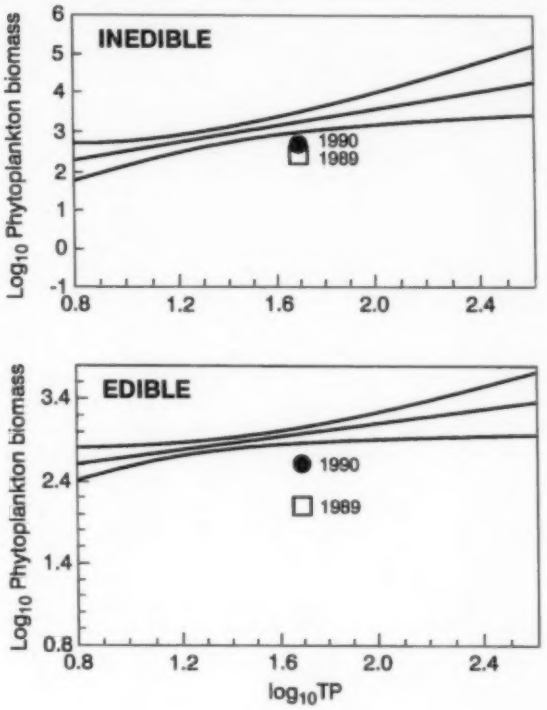

Table 5. Comparison of predicted versus actual estimates of zooplankton biomass (in $mg \cdot m^{-3}$ wet (algal biomass model) or dry weight) in Redberry Lake according to various regression models.

		Zooplankton biomass		
Year and model	Value used	Predicted	Actual	Ratio
Total phosphorus[a]				
1989	47.0	187.3	211.0	1.1
1990	47.2	188.1	340.2	1.8
Algal biomass[b]				
1989	654.5	1083	2110	2.0
1990	738.2	1181	3400	2.8
Chlorophyll[a]				
1989	1.71	36.6	211.0	5.8
1990	1.62	35.6	340.0	9.6

Note: Ratio are actual/predicted values.
[a]Model from Hanson and Peters (1984).
[b]Model from McCauley and Kalff (1981).

estimated the percent chlorophyll content of the phytoplankton inhabiting Campbell and Prepas's study lakes first by using Watson and McCauley's (1988) regression to predict algal biomass in these lakes. Next, we used Campbell and Prepas' predictions of chlorophyll concentration in their lakes; predictions were based on Smith's (1982) model, which considers

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both TP and TN. Mean percent chlorophyll content was then estimated by dividing estimated mean chlorophyll concentration by estimated biomass and multiplying the result by 100. Values were 0.05% for Oliva Lake, 0.07% for Peninsula Lake, and 0.09% for Fleuvog Lake. We used these values, rather than the 1% estimate used by Campbell and Prepas, to estimate phytoplankton biomass based on actual chlorophyll determinations. Predicted zooplankton biomass, based on McCauley and Kalff's (1981) model, was 3530 $mg \cdot m^{-3}$ for Fluevog Lake, 10 055 $mg \cdot m^{-3}$ for Peninsula Lake, and 6627 $mg \cdot m^{-3}$ for Oliva Lake or 3.7, 1.4, and 12.4 times higher, respectively, than model predictions developed for freshwater systems.

In contrast to Campbell and Prepas' (1986) study and our Redberry Lake study, we did not observe that zooplankton biomass was relatively high when compared with phytoplankton biomass in Humboldt Lake (Evans et al. 1995). In fact, zooplankton biomass was 3.9–5.0 times lower than model predictions based on algal biomass. We attributed this observation to the fact that intense zooplankton grazing in Humboldt Lake had resulted in a zooplankton community in which edible algae accounted for a very small percentage of algal biomass.

The phytoplankton/zooplankton biomass ratio for Redberry Lake was 0.31 in 1989 and 0.22 in 1990; predicted ratios were 0.60 and 0.63, respectively. Estimated phytoplankton/zooplankton biomass ratios (see above) for Fluevog, Peninsula, and Oliva lakes were 0.26, 1.02, and 0.10 versus predicted values of 0.94, 1.42, and 1.21, respectively. Actual values, while low, were within the range of ratios implicit in McCauley and Kalff's (1981) zooplankton biomass model (Table 6). This ratio may increase with increasing algal biomass because as algal standing stocks increase with increasing TP, inedible algae account for an increasingly large fraction of total algal biomass; however, production rates of inedible and edible algae increase at a similar rate (Watson and McCauley 1988). Moreover, there is some evidence that the relationship between phytoplankton and zooplankton biomass differs between freshwater and saline lake systems with differences being particularly large for the Alberta lakes investigated by Campbell and Prepas (1986).

Chlorophyll was the poorest predictor of zooplankton biomass in Redberry Lake, with zooplankton 5.8–9.6 times more abundant than predicted by McCauley and Kalff's (1981) model. Similarly, Hanson and Peters' (1984) chlorophyll model underestimated zooplankton biomass in Campbell and Prepas' (1986) study lakes by a factor of approximately 100. Chlorophyll also underestimated zooplankton biomass in Humboldt Lake by a factor of 4.3–4.5 (Evans et al. 1995). We attributed this observation, in part, to the fact that Hanson and Peters' models were developed for freshwater lakes where the chlorophyll content of algal cells may approach the 1% value suggested by Ahlgren (1970). The chlorophyll content of algal cells inhabiting saline lakes may be lower and thus algal biomass may be higher than inferred from chlorophyll determinations.

Table 6. Predicted zooplankton biomass and phytoplankton/zooplankton biomass ratios based on McCauley and Kalff's (1981) phytoplankton–zooplankton biomass (wet weight) model.

Phytoplankton biomass ($\mu g \cdot L^{-1}$)	Predicted zooplankton biomass ($\mu g \cdot L^{-1}$)	Phytoplankton/zooplankton biomass
10	54	0.19
100	284	0.35
1 000	1 486	0.67
10 000	7 780	1.29
100 000	40 730	2.45

Chlorophyll and algal biomass

We estimated that the chlorophyll content of algal cells in Redberry Lake ranged from 0.10 to 0.69% and averaged 0.40%. This is lower than the 1% value that Ahlgren (1970) suggested was a representative chlorophyll to algal biomass conversion factor. Thus, the use of this 0.01 conversion factor to estimate algal biomass on the basis of chlorophyll determinations will underestimate algal biomass. For example, in Redberry Lake, chlorophyll concentrations averaged 1.71 $\mu g \cdot L^{-1}$ in 1989 and 1.62 $\mu g \cdot L^{-1}$ in 1990. The use of the 0.01 conversion factor results in mean estimates of algal biomass of 171 and 162 $\mu g \cdot L^{-1}$, respectively, or 3.8–4.6 times lower than actual values. In Humboldt Lake, we estimated that the mean chlorophyll content of algal cells averaged 0.05% in 1989 and 0.03% in 1990 (Evans et al. 1995). Algal biomass was 20–33 times greater than inferred from chlorophyll concentration and the use of the 0.01 conversion factor.

In our Humboldt Lake study, we estimated the theoretical mean percent chlorophyll content of algal cells from Watson and McCauley's (1988) TP – algal biomass models and Smith's (1982) chlorophyll–TP–TN models (Evans et al. 1995). If we assume that Redberry Lake has an ice-free season average TN/TP ratio of 1, the predicted percent chlorophyll content of algal biomass is 0.08%. If we assume that the TN/TP ratio is 9, the predicted percent chlorophyll content becomes 0.38%, which is close to observed values.

Zooplankton and phytoplankton in Redberry Lake

Overall, our research has shown that zooplankton biomass is relatively high when compared with algal biomass in Redberry Lake. Several factors could account for this. The high biomass of zooplankton may be related to the fact that planktivorous fish are generally absent from this lake. Thus, zooplankton mortality rates resulting from fish predation are low. Moreover, the zooplankton community is relatively simple and energy may be transferred efficiently from one trophic level to another.

Redberry Lake has a relatively simple zooplankton community dominated by *Diaptomus sicilis* and *Daphnia pulicaria*, both herbivorous species. *Diaptomus sicilis* feeds most efficiently on particles 10–40 μm in size (Vanderploeg 1981) whereas *Daphnia pulicaria* has a lower preferred size range and is able to filter particles as small as bacteria (Gliwicz 1980; Knoechel and Holtby 1986). This relatively simple community may allow for an efficient transfer of trophic energy from the phytoplankton to zooplankton communities. Similarly, the zooplankton communities in the lakes investigated by Campbell and Prepas (1986) were dominated by herbivores and food energy may have been transferred efficiently from the phytoplankton to zooplankton communities.

As in Campbell and Prepas (1986), primary production appears to be insufficient to meet the needs of the zooplankton community in Redberry Lake. We used two approaches to

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estimate zooplankton carbon requirements and, ultimately, to assess whether or not primary production was sufficient to meet these needs. First, we used Parsons et al.'s (1984) estimate that small crustacean zooplankton ingest 40–60% of their body weight per day. Using the lower figure, Redberry Lake zooplankton may ingest an average of 84.4–136.1 $mg \cdot m^{-3}$ of dry weight of algal biomass per day. Assuming an algal dry weight to wet weight conversion factor of 5 (Strickland 1960), this ingestion rate corresponds to 6.8–10.9 $g \cdot m^{-2}$ of algal biomass (wet weight) per day for a 16 m deep water column. Again, assuming a 0.13 algal biomass to carbon conversion factor, this represents a daily ingestion rate of 0.9–1.4 $g\ C \cdot m^{-2} \cdot day^{-1}$. We also used Campbell and Prepas' (1986) estimate that zooplankton require 0.80 $\mu g\ C \cdot \mu g^{-1} \cdot day^{-1}$ (dry weight basis), resulting in a higher ingestion rate estimate of 2.7–4.4 $g\ C \cdot m^{-2} \cdot day^{-1}$. In contrast, the average daily primary production rate for Redberry Lake was 0.46 $g\ C \cdot m^{-2} \cdot day^{-1}$ for the 2 years of study (Robarts et al. 1992). Thus, zooplankton carbon requirements appear to be 5.9–9.6 times higher than the estimated 2-year, average daily primary production rates for Redberry Lake.

Zooplankton inhabiting Redberry Lake must utilize other strategies for maintaining their high biomass relative to their apparent food requirements. For example, during summer, zooplankton may reduce their metabolic rates by inhabiting the hypolimnion during a significant proportion of the day (McLaren 1963: Vanderploeg 1992). Energetic demands may also be reduced during the colder months of the years. Zooplankton food demands may also be met through phagotrophic algal production. While we did not measure phagotrophic production, many of the dominant (biomass basis) phytoplankton in Redberry Lake during the ice-free season were taxa capable of such production, i.e., flagellates, chrysophytes, and dinoflagellates. Ciliates also were present, although they generally did not constitute a significant proportion of total algal biomass. Where energetic demands are not met and lipid reserves insufficient, zooplankton may experience significant mortality rates (Arts et al. 1993; M.S. Evans, unpublished data).

Concluding remarks

Overall, our research on Redberry Lake and Humboldt Lake (Evans et al. 1995), and Campbell and Prepas' (1986) investigation of three saline Alberta lakes indicate that algal production is much lower in these systems than expected on the basis of their TP concentrations. Algal biomass and chlorophyll concentrations in Redberry Lake and chlorophyll concentrations in Campbell and Prepas' lakes also are lower than expected for their TP concentrations. However, algal biomass in Humboldt Lake was higher than Watson and McCauley's (1988) model predictions. It is possible that, with a salinity of 3 $g \cdot L^{-1}$, Humboldt Lake functions more like a freshwater lake than a saline lake. We conducted a similar algal biomass – TP study in Lenore Lake, another saline prairie lake. Lenore Lake has a specific conductivity of ca. 6500 $\mu S \cdot cm^{-1}$, a salinity of ca. 6.9 $g \cdot L^{-1}$, and a mean TP concentration of 32 $\mu g \cdot L^{-1}$. Like Humboldt Lake, TP underestimated algal biomass (M.S. Evans, unpublished data). Thus, it may only be when salinities approach 9000 $S \cdot cm^{-1}$ that TP overestimates both algal production and biomass. More research needs to be conducted over a wider range of salinities and salinity types (sodium versus magnesium sulfate lakes) to determine consistent patterns in deviations between model predictions and actual determinations of biomass and production with increasing salinity.

Our study has shown that Redberry Lake maintains high water clarity despite TP concentrations that are typical of eutrophic to hypereutrophic lakes. Moreover, the water column remains well oxygenated with nutrient and particulate concentrations in the deep layer only slightly higher than in the surface layer. The lake is productive, with a mean daily production rate of 462 $mg\ C \cdot m^{-2} \cdot day^{-1}$ for the 2 years of study (Robarts et al. 1992); this rate is typical of oligomesotrophic lakes (Wetzel 1983). Moreover, algal carbon turnover times owing to primary production are short, suggesting rapid growth rates. Nevertheless, chlorophyll concentrations and algal biomass remain low. Our data suggest that zooplankton grazing is a significant factor affecting the low algal biomass in Redberry Lake and, ultimately, depresses total annual production.

While our research suggests that zooplankton grazing is an important factor contributing to the low biomass of phytoplankton in Redberry Lake, other studies suggest that additional factors may be as important. Waiser and Robarts (1995) determined that the majority of the measured soluble reactive phosphorus in Redberry Lake is not available for algal growth and hypothesized that high levels of DOC modified phosphorus availability. However, their data also showed that phytoplankton generally were P sufficient with moderate P deficiency occurring only in July, August, and October. In addition, phytoplankton photosynthetic capacity was high (ca. 10–17 $mg\ C \cdot mg\ chlorophyll^{-1} \cdot h^{-1}$) during the warmer (15–22°C) months of the year, suggesting that light and nutrients were not limiting (Robarts et al. 1992). Phagotrophic uptake of phosphorus may provide an alternative route for phosphorus uptake (Waiser and Robarts 1995). Thus, the phytoplankton assemblage may not be as phosphorus deficient as inferred from the reduced availability of soluble reactive phosphorus.

In summary, while our research has elucidated some of the factors affecting the high water clarity in Redberry Lake despite mean TP concentrations of ca. 50 $\mu g \cdot L^{-1}$, it also has illuminated additional puzzles. By inference, these puzzles apply to other saline prairie lake ecosystems. Continuing research on saline prairie lakes will further elucidate these differences and suggest ways in which existing regression models developed for freshwater systems can be further refined in their predictive capabilities for these ecosystems.

Acknowledgments

Eric Marles, Alan Merkowsky, Marla Nahachewsky, Pat Shaw, and Marley Waiser participated in the field sampling and laboratory studies. Dr. J.C. Earle (Concordia College, Edmonton, Alberta) provided expert identification and quantification of seasonal changes in phytoplankton species composition and biomass. Pat Shaw and David Peters developed the computer program used in enumerating and measuring the zooplankton. Vijay Tumber prepared the figures. The manuscript benefited from comments provided by Dr. E. Prepas (University of Alberta, Edmonton) and Dr. K.E. Havens

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(South Florida Water Management District, West Palm Beach, Florida).

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Stream-bed scour, egg burial depths, and the influence of salmonid spawning on bed surface mobility and embryo survival

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Abstract: Bed scour, egg pocket depths, and alteration of stream-bed surfaces by spawning chum salmon (*Onchorhynchus keta*) were measured in two Pacific Northwest gravel-bedded streams. Close correspondence between egg burial depths and scour depths during the incubation period suggests an adaptation to typical depths of bed scour and indicates that even minor increases in the depth of scour could significantly reduce embryo survival. Where egg burial depths are known, expressing scour depth in terms of bed-load transport rate provides a means for predicting embryo mortality resulting from changes in watershed processes that alter shear stress or sediment supply. Stream-bed alteration caused by mass spawning also may influence embryo survival. Theoretical calculations indicate that spawning-related bed surface coarsening, sorting, and form drag reduce grain mobility and lessen the probability of stream-bed scour and excavation of buried salmon embryos. This potential feedback between salmon spawning and bed mobility implies that it could become increasingly difficult to reverse declines in mass-spawning populations because decreased spawning activity would increase the potential for bed scour, favoring higher embryo mortality. Further analysis of this effect is warranted, however, as the degree to which spawning-related bed loosening counteracts reduced grain mobility caused by surface coarsening, sorting, and redd form drag remains uncertain.

Résumé : Des chercheurs ont mesuré l'affouillement du lit, la profondeur de la ponte et l'altération de la surface du lit de gravier de deux cours d'eau causée par le saumon kéta (*Onchorhyncus keta*) sur la côte nord-ouest du Pacifique. L'étroit rapprochement entre la profondeur d'enfouissement des oeufs et celle de l'affouillement pendant la période d'incubation pourrait pointer vers une adaptation à des profondeurs caractéristiques d'affouillement et pourrait signifier que même un léger approfondissement de l'affouillement pourrait nuire sensiblement à la survie des embryons. Lorsqu'on connaît la profondeur d'enfouissement des oeufs, l'expression de la profondeur d'affouillement en termes de charge de fond constitue un moyen de prévoir la mortalité des embryons attribuable à des changements dans les conditions dynamiques du bassin hydrographique à l'origine d'une modification de la force de cisaillement exercée par l'eau ou de l'apport en sédiments. Altération de la surface du lit de gravier causée par l'activité de fraye en masse influence peut-être la survie des embryons. Des calculs théoriques indiquent que la rugosité du lit, que le tri des matériaux à sa surface ainsi que la microtopographie des nids, attribuables à l'activité de fraye des saumons, devraient diminuer la mobilité des grains et abaisser la probabilité d'un affouillement du lit ainsi que de l'excavation des embryons de saumon enfouis. L'existence possible d'une telle boucle de rétroaction entre la fraye du saumon et la mobilité du lit signifie qu'il pourrait être de plus en plus difficile de renverser la tendance chez des populations en déclin qui fraient en masse puisque toute diminution de l'activité de fraye devrait correspondre à une hausse de la possibilité pour l'affouillement des nids, ce qui favorise une hausse du taux de mortalité chez les embryons. L'analyse plus poussée de cet effet paraît justifiée puisqu'on ignore dans quelle mesure l'ameublissement du lit associé à la fraye enraye la perte de mobilité des grains attribuable au durcissement de la surface du lit, au tri et à la traînée causée par la forme du nid.
[Traduit par la Rédaction]

Introduction

Dramatic declines of Pacific salmon (*Oncorhynchus* spp.) in the northwestern United States led to the application of the Endangered Species Act to certain populations (Federal Register 1991, 1992) and concern that many other populations are either in jeopardy or already extinct (Nehlsen et al. 1991). Declines in salmon populations reflect the combined influences of dam construction, overfishing, habitat degradation, and other factors, but it has proven extremely difficult to isolate specific influences, in part because of difficulties in linking habitat change to population-level response (Holtby and Scrivener 1989; Bisson et al. 1992). We hypothesize that an important factor in salmon population biology is the active role that salmon play in the creation and maintenance of their own habitat. Beaver dams are probably the most widely recognized

Received November 11, 1994. Accepted November 20, 1995.
J12628

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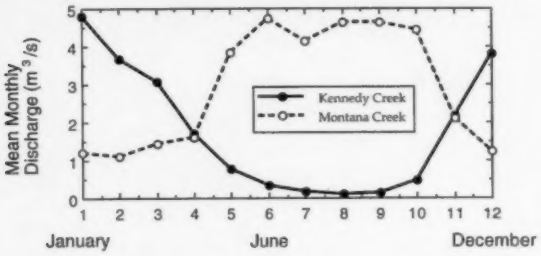
Fig. 1. Annual hydrographs of mean monthly flows for Kennedy Creek (1960–1971) and Montana Creek (1965–1987).



example of animal–habitat interactions (e.g., Ives 1942; White 1979; Naiman et al. 1988), but burrowing activity (e.g., Grinnell 1923; Cox 1987; Black and Montgomery 1991) and grazing (e.g., McNaughton et al. 1988) provide additional examples. It is well known that spawning salmonids construct redds for their eggs and that in the process they sort and coarsen stream-bed gravels (e.g., Everest et al. 1987; Chapman 1988; Young et al. 1989; Kondolf et al. 1993) and influence the invertebrate community (Field-Dodgson 1987), but neither fisheries biologists nor geomorphologists have explored how spawning activity influences bed mobility and reproductive success.

Anadromous salmon are spawned in fresh water, migrate to sea after a variable period of freshwater residence, spend several years at sea, then return to their natal stream and spawn. Each female constructs a redd consisting of several egg pockets dug in the channel bed by turning on her side and rapidly undulating her tail and body. This action sweeps finer particles into the water column where they are transported downstream (e.g., Kondolf et al. 1993). After eggs are released and fertilized, the female buries them while simultaneously excavating another egg pocket immediately upstream (Groot and Margolis 1991). So long as they are alive, females defend these nests from encroachment, but high spawning densities may result in superimposition of nests, which is widely accepted as a major cause of density-dependent mortality (Hunter 1959; McNeil 1964). In the absence of substantial superimposition, most embryo mortality is thought to occur from poor water circulation associated with the filling of intergravel pore spaces by fine sediment (Tappel and Bjornn 1983; Chapman 1988; Lisle and Lewis 1992) and scour during high-flow events (McNeil 1964; Holtby and Healey 1986; Lisle 1989). Although high salmon densities can reduce survival, we report evidence that mass spawning activity alters channel morphology in ways that may reduce the vulnerability of eggs to scour. We further show that nested embryos are quite vulnerable to increases in the average depth of bed scour.

Study areas

The study sites are low-gradient, gravel-bedded channels used by spawning chum salmon (*Oncorhynchus keta*). Kennedy Creek is located at the south end of Puget Sound, Washington, and Montana Creek is near Juneau, Alaska. The Kennedy Creek study reach is a pool–riffle type channel with a bank-full width and depth of approximately 10.8 and 0.42 m, and a reach-average slope of 0.0047. Flow convergence and divergence associated with accumulations of large woody debris (LWD) locally influence the bed morphology. The Montana Creek study reach is a plane-bed type channel (Montgomery and Buffington 1993) with a bank-full width and depth of 18.4 and 0.72 m, and a reach-average slope of 0.0037. The Montana Creek reach has little cross-channel topography and a very low LWD loading (i.e., 0.01 pieces/m^2). The annual hydrograph of Kennedy Creek is rainfall dominated with the greatest average discharges occurring from November through March (Fig. 1), a period coinciding with incubation of salmonid embryos in the stream-bed gravel. In contrast, summer high flows dominate the annual hydrograph of Montana Creek, with a relatively uniform high discharge period from May through October (Fig. 1). Chum salmon typically spawn in Montana Creek during July in the middle of this high discharge period.

Methods

Paired pebble counts (Wolman 1954) of 100 grains were conducted in each reach in unspawned locations proximal to redds and in tailspill or tailout portions of redds immediately downstream of egg pockets. Redds (spawned sites) were readily discerned by a change in the texture and patina of the bed surface. Pebble counts were conducted during spawning activity in the fall of 1991 in Kennedy Creek and in the summer of 1993 in Montana Creek. A pebble count (Wolman 1954) records the intermediate axis length of clasts randomly selected during a traverse across the channel bed. Characterization of the bed surface material is preferable to bulk samples of the channel substrate for examining bed stability because the size of material in the surface layer governs bed mobility.

Channel cross sections and slopes were surveyed using a digital theodolite in Kennedy Creek and a hand level and stadia rod in Montana Creek. Redd dimensions and frequencies estimated at each study site characterized alteration of bed topography during spawning activity. Repeated topographic mapping of the entire study reach immediately prior to and after spawning further quantified effects on channel morphology at Kennedy Creek.

A total of 104 scour chains placed throughout the Kennedy Creek site gauged the depth of bed scour and fill during the winter season following spawning. Scour monitors consisted of an anchored chain with a series of 4 cm diameter perforated plastic balls, a variant on the sliding bead scour monitors developed by Nawa and Frissell (1993). Scour monitors were distributed throughout the study reach both at redd locations and at sites not used by spawning fish. Egg pocket depths in 40 redds were measured after visual identification immediately following spawning (see Peterson and Quinn (1996) for further details). Field observations of discharge and channel scour at Kennedy Creek were made from November 1991 to February 1992 during the time of both peak flows and incubation of salmonid embryos within stream gravels.

Results

Spawning occurred throughout the entire study reach at Kennedy Creek during fall 1990, coarsening the bed surface, partially filling pools, and excavating bar margins. Similar spawning effects on channel morphology were documented the following year through repeated topographic surveying before and after spawning activity. Redds formed a distinctive microtopography on the channel bed with an amplitude of 10–20 cm and a wavelength of about 2.0 m. These bed forms persisted until the bed surface was reworked during high winter flows. At Montana Creek, half of the bed surface had been disturbed by spawning at the time of the survey. Redd

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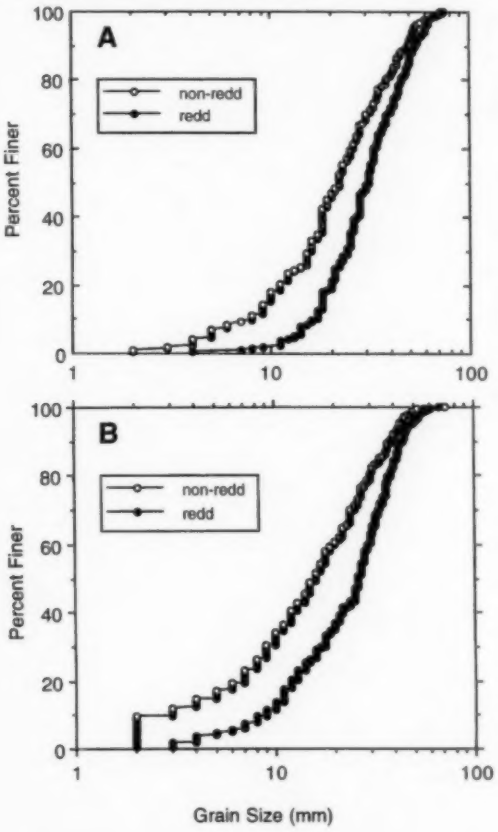
Table 1. Median surface grain sizes and sorting coefficients of paired unspawned and spawned samples.

Sample	d_{50} (mm)			σ_I (ϕ)		
	Unspawned	Spawned	% difference	Unspawned	Spawned	% difference
			Kennedy Creek			
1	23	32	+39	1.18	0.61	–48
2	21	28	+33	0.94	0.75	–20
			Montana Creek			
1	12	20	+67	1.70	0.93	–45
2	16	25	+56	1.35	1.18	–13
3	15	26	+73	1.48	0.70	–53
4	18	29	+61	1.11	0.99	–11

Note: σ_I is the inclusive graphic standard deviation defined as $(\phi_{95} - \phi_{16})/4 + (\phi_{95} - \phi_5)/6.6$ (Folk 1974), where ϕ is the standard log base 2 unit of grain size measurement and the subscripts represent percentiles of a given grain size distribution in ϕ units. Lower values of σ_I indicate better sorting.

Fig. 2. (A) Composite cumulative size distributions of bed surface sediment for non-redd and redd locations sampled on October 28, 1991, at Kennedy Creek. (B) Composite cumulative size distributions of bed surface sediment for non-redd and redd locations sampled on July 21 and 22, 1993, at Montana Creek.



microtopography was comparable to that observed in Kennedy Creek.

Bed-surface grain size

Spawning activity visibly coarsened the channel bed in both study reaches, as demonstrated by grain size distributions of unspawned and postspawning samples (Table 1). Spawning caused a 33–39% increase in median surface grain size d_{50} in Kennedy Creek, and a 56–73% increase in d_{50} in Montana Creek. While the coarse end of each composite grain size distribution was similar, the percentage of fine particles on the redd surfaces decreased (Fig. 2). Body undulations during redd construction cause fine particles to be suspended and transported downstream (e.g., Kondolf et al. 1993), producing coarser and better sorted gravels. Fine sediment from portions of the bed surface disturbed by spawning in Kennedy Creek collected downstream on channel margins and in pools. Bed surface sorting, defined by the inclusive graphic standard deviation (Folk 1974), σ_I, increased by 20–48% in Kennedy Creek and by 11–53% in Montana Creek as a result of spawning (Table 1).

Table 2. *t* tests of all possible groupings of spawned (S) and unspawned (U) samples from Kennedy Creek.

Sample grouping	Probability of similarity
1U/2U	0.528
1U/1S	0.000
1U/2S	0.016
2U/1S	0.000
2U/2S	0.002
1S/2S	0.083

Note: Unpaired, two-tailed *t* tests were used assuming unequal and unknown variances.

Statistical similarity among the mean grain sizes of spawned and unspawned portions of Kennedy Creek was evaluated through *t* tests (Table 2). Differences among unspawned samples were not significant at a 0.05 probability level. The same was true of postspawning samples. However, differences between spawned and unspawned grain size distributions were significant ($P < 0.05$). Composite grain size distributions indicate that spawning activity locally increased d_{50} from 22 to 30 mm in Kennedy Creek (Fig. 2).

Similarity among the postspawning and unspawned locations in Montana Creek also was evaluated using *t* tests (Table 3). While each of the paired samples differed significantly, one of the postspawning samples (1S) was not different from the unspawned samples of the other paired pebble counts (2U, 3U, and 4U). Except for sample 1, all spawned samples were similar, as were all unspawned samples. These observations

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Table 3. *t* tests of all possible groupings of spawned (S) and unspawned (U) samples from Montana Creek.

Sample grouping	Probability of similarity
1S/2S	0.016
1S/3S	0.003
1S/4S	0.000
2S/3S	0.988
2S/4S	0.316
3S/4S	0.238
1U/2U	0.015
1U/3U	0.051
1U/4U	0.015
2U/3U	0.682
2U/4U	0.888
3U/4U	0.770
1S/1U	0.000
1S/2U	0.290
1S/3U	0.144
1S/4U	0.196
2S/1U	0.000
2S/2U	0.001
2S/3U	0.000
2S/4U	0.000
3S/1U	0.000
3S/2U	0.000
3S/3U	0.000
3S/4U	0.000
4S/1U	0.000
4S/2U	0.000
4S/3U	0.000
4S/4U	0.000

Note: Unpaired, two-tailed *t* tests were used assuming unequal and unknown variances.

are consistent with Kondolf et al.'s (1993) finding that the best predictor of the postspawning concentration of fine sediment in redds is the prespawning concentration. Composite grain size distributions from Montana Creek showed that d_{50} increased from 15 mm before spawning to 25 mm after spawning (Fig. 2).

In addition to coarsening and sorting bed surface grains, spawning activity also changed bed material packing. Although we did not quantify bed packing, we noted that in both Kennedy Creek and Montana Creek, gravels disturbed by spawning were more loosely packed than unspawned portions of the bed. Postspawning gravels were typically overloose in the terminology introduced by Church (1978), whereas prespawning gravels were underloose (imbricated) to normally loose. Application of these qualitative terms, however, is highly subjective.

Bed scour and egg pocket depths

The peak discharge observed in Kennedy Creek from November 1991 to February 1992 slightly exceeded bank-full stage. During this approximately bank-full event, scour depths recorded by 104 scour chains distributed throughout the reach exhibited a roughly exponential distribution ranging from 0 to 60 cm, with a mean of 13.4 cm (Fig. 3A). Forty measured egg-pocket depths ranged from 9.8 to 48.9 cm, with a mean of 22.6 cm (Fig. 3B). Comparison of bed scour and egg pocket

Fig. 3. Normalized histographs of (A) scour and (B) egg burial depths for Kennedy Creek in 1991–1992. Scour depths (n = 104) were determined from scour chains monitored through the winter season. Egg burial depths (n = 40) were measured during spawning activity.

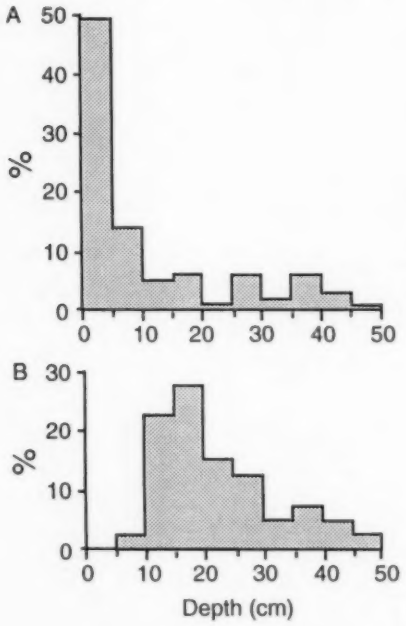
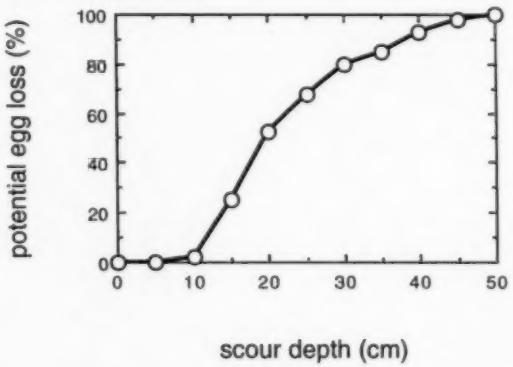


Fig. 4. Percent survival versus scour depth for Kennedy Creek derived from Fig. 3B.



distributions revealed that only a small proportion of the channel bed scoured to egg-pocket depths during this approximately bank-full event. Sixty-five percent of the scour chains recorded scour of less than 10 cm, while less than 5% of the egg pockets were less than 10 cm deep. Distributions of scour and egg-pocket burial depths, however, indicated that even a slight increase in scour depths would significantly affect the integrity of many egg pockets. A doubling of the mean scour depth to 26.8 cm, for example, would jeopardize the majority of egg pockets (Fig. 4).

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Discussion

Salmonid reproductive success depends in part on egg burial depths exceeding the depth of scour during the incubation period. Successful reproduction thus requires adjustment to the typical timing and depth of bed scour. The observed distribution of egg burial depths below scour depths during a bank-full flood both suggests such an adjustment and indicates that an increase in the average depth of bed scour would significantly affect salmon populations. A strong correlation between mortality and peak seasonal discharge (Holtby and Healey 1986) suggests a direct link to bed scour. Therefore, conceptual models that relate bed scour to discharge and bed-load transport rates provide a framework for predicting embryo mortality caused by changes in watershed processes and grain size.

Bed scour

The depth of scour in gravel-bedded channels reflects the rate of bed-load transport, which is a function of boundary shear stress (and thus flow stage) relative to the critical shear stresses of both the surface and subsurface material (e.g., Emmett and Leopold 1963; Leopold and Emmett 1984; Carling 1987; Hassan et al. 1991). In armored gravel-bedded streams, mobility of the surface layer is followed by a rapid rise in bed-load transport (Jackson and Beschta 1982) resulting from exposure and scour of the finer and more easily mobilized subsurface material. The rate of bed-load transport is also a function of sediment supply. Increased sediment loads cause bed surface fining and thus higher transport rates for a given shear stress (e.g., Dietrich et al. 1989). Finer bed surfaces likely cause more frequent and greater depths of scour.

The depth of scour can be determined from the bed-load transport rate as

$$(1) \quad d_s = Q_b/(u_b \rho_s (1 - y))$$

where d_s is the mean depth of scour, Q_b is the bed-load transport rate per unit channel width, u_b is the average bed-load velocity, ρ_s is sediment density, and y is bed porosity (Carling 1987). Bed-load transport rates vary substantially between channels and thus primarily control average scour depths.

Numerous expressions describe bed-load transport as a nonlinear function of shear stress, discharge, velocity, or stream power (see review by Gomez and Church 1989). One conceptually simple equation expresses the bed-load transport rate per unit channel width as a function of the difference between the effective basal shear stress (τ') and the critical shear stress (τ_c):

$$(2) \quad Q_b = k(\tau' - \tau_c)^{1.5}$$

where k is an empirical constant. Together eqs. 1 and 2 provide a simple framework for quantifying scour depth and potential embryo mortality owing to changes in watershed processes. Changes in either the effective or critical shear stresses can alter bed-load flux and should influence the depth of scour and thereby embryo survival. Changes in sediment supply can alter τ_c (e.g., Dietrich et al. 1989), while changes in discharge and LWD loading can affect τ' (e.g., Buffington 1995). Furthermore, the observed stream-bed modifications caused by spawning chum salmon also have the potential to alter both τ' and τ_c and thus influence bed scour and embryo mortality.

Influence of spawning on stream-bed mobility

Salmonid-induced modifications of grain size, sorting, packing, and bed topography all potentially affect bed surface mobility, and thereby the vulnerability of embryos to fluvial excavation. In gravel-bedded channels of mixed grain sizes, the mobility of the median surface grain size provides an indicator of general bed mobility. Many gravel-bedded rivers exhibit equal grain mobility at incipient motion (Parker et al. 1982), owing in part to surface armoring (Jackson and Beschta 1982), friction angle distributions (Buffington et al. 1992), and hiding effects (Einstein 1950), which together cause a threshold condition of general bed surface mobility. Consequently, the critical boundary shear stress required to mobilize the surface grains of a gravel-bedded channel can be estimated from Shields' (1936) equation written in terms of the surface d_{50}:

$$(3) \quad \tau_{c50} = \tau^*_{c50}(\rho_s - \rho_w)g d_{50}$$

where τ_{c50} and τ^*_{c50} are the dimensional and dimensionless critical shear stresses for the median surface grain size, ρ_s and ρ_w are the densities of sediment and water, and g is gravitational acceleration. Numerous and disparate values reported in the literature complicate determining τ^*_{c50} (see reviews by Church 1978 and Buffington 1995). Here we use Parker and Klingeman's (1982) value of 0.035, as it represents bed surface characteristics of mixed grain sizes typical of gravel-bedded streams and is derived from a study reach with subdued bedform roughness (Milhous 1973). Regardless of the specific τ^*_{c50} value used, inspection of eq. 3 shows that τ_{c50} is linearly proportional to d_{50}, implying that surface coarsening increases the shear stress necessary to initiate general bed mobility.

Incorporating sediment and water densities of 2650 and 1000 kg/m^3 into eq. 3 predicts that spawning-related bed coarsening (i.e., 22–30 mm) increased the critical shear stress from 12.5 to 17.0 Pa in Kennedy Creek. Comparison of these shear stresses with the bank-full shear stress allows the significance of this increase in τ_{c50} to be estimated. The reach-average bank-full shear stress calculated from the depth–slope product ($\tau_0 = \rho_w g D S$, where τ_0 is the total boundary shear stress and D and S are the flow depth and slope, respectively) is approximately 19.4 Pa. This predicts that spawning-related surface coarsening therefore increased the critical shear stress from about 64 to 88% of the bank-full shear stress. However, these theoretical (i.e., calculated) critical shear stresses underestimate the effects of bar form and LWD roughness that dissipate shear stress, effectively lowering the basal shear stress available for sediment transport (i.e., τ') and increasing the total shear stress required for grain mobility (see Buffington 1995 for further discussion). Hence, τ_{c50} for redd surfaces is likely to exceed 88% of the bank-full shear stress.

Similar critical shear stress calculations for Montana Creek indicate that spawning-related bed coarsening increased τ_{c50} from approximately 33 to 54% of the bank-full shear stress. Roughness effects associated with LWD and bar forms are minimal in Montana Creek. Equation 3 thus predicts that spawning-related bed surface coarsening significantly increases the critical shear stress in both Kennedy and Montana creeks.

Sorting of bed surface material during spawning likely further reduces grain mobility. Increased sorting raises friction angles (Φ) between the bed surface and particles moving over the bed (Fig. 5), causing greater resistance to motion (Miller

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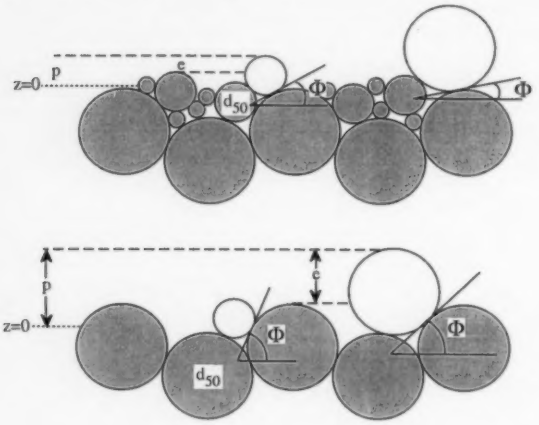
Fig. 5. Diagram illustrating the increase in friction angles, Φ, owing to increased sorting. Grains composing the bed surface are shaded, while particles traversing the bed are unshaded. Removal of finer grains during spawning activity produces better sorted bed surfaces that present deeper intergranular pockets and higher friction angles. Protrusions of particles equivalent in size to the d_{50} of the bed surface are also shown. Protrusion is defined by a particle's projection, p, above the local datum, $z = 0$, and by its exposure, e, above upstream grains (Kirchner et al. 1990).



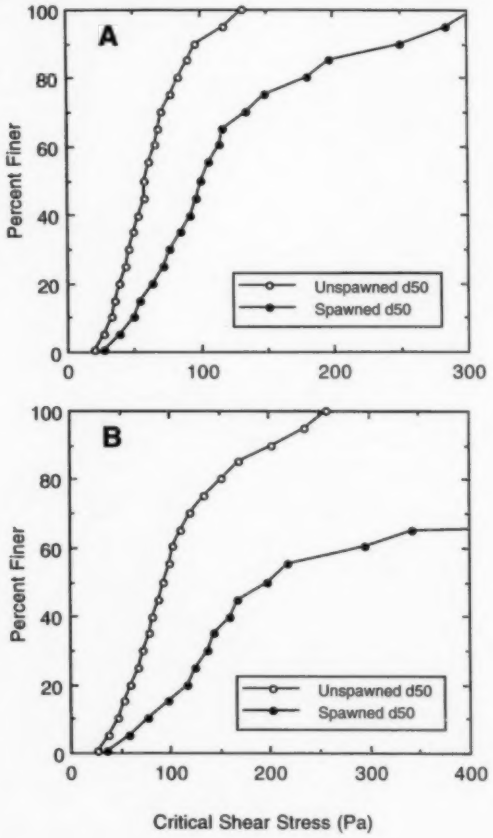
and Byrne 1966; Buffington et al. 1992). The varied arrangement of grain sizes composing a heterogeneous bed surface results in a distribution of friction angles for a given particle size on that surface (Kirchner et al. 1990; Buffington et al. 1992). Distributions of Φ for unspawned and spawned values of d_{50} were determined empirically as functions of grain size and sorting (σ_I) (Buffington et al. 1992). In addition to friction angle, grain mobility depends on projection (p) and exposure (e) (Fig. 5) (Fenton and Abbott 1977). As with Φ, distributions of p and e characterize each particle size on a bed surface and can be determined empirically as functions of grain size and Φ (Kirchner et al. 1990).

The combined effects of bed surface coarsening and sorting on grain mobility are examined by calculating critical shear stresses of unspawned and postspawning gravels using Kirchner et al.'s (1990) theoretical critical shear stress formulation. In this model, the critical shear stress for incipient grain motion is expressed as

$$\tau_c = (\rho_s - \rho_w)\, g\pi d^3 \kappa^2 (\tan\Phi\cos\alpha - \sin\alpha) \Big/ 3\left(C_D \int_{p-e}^{p} f^2(z)(d^2 - (2z - (2p - d))^2)^{1/2}\, dz + \frac{C_L \pi d^2}{4} f^2(z)\Big|_{p-d}^{p} \tan\Phi\right) \quad (4)$$

where d is grain size, von Kármán's constant κ equals 0.407 (Wiberg and Smith 1987), α is the inclination of the bed, C_D (≈0.45) is the drag coefficient for a sphere at high-particle Reynolds numbers (i.e., 10^3–10^5) (Rouse 1946), z is the height above the bed, $f(z)$ is a velocity profile function of the law of the wall, and C_L (≈0.2) is the lift coefficient for a sphere (Wiberg and Smith 1987) (see Kirchner et al. 1990 for further details). Inspection of eq. 4 shows that τ_c is inversely proportional to grain protrusion ($p - e$), and directly proportional to d and Φ (and thus σ_I).

Fig. 6. Calculated critical shear stress distributions of d_{50} grain sizes on unspawned and spawned bed surfaces in (A) Montana Creek and (B) Kennedy Creek.



Results from this analysis predict a shift in the τ_{c50} distributions for unspawned and postspawning surfaces (Fig. 6). In Montana Creek the median value of τ_{c50} increases by 73% as a result of surface coarsening and sorting. Moreover, there is a threefold increase in the overall range of stresses required to entrain d_{50} grain sizes in Montana Creek. In Kennedy Creek the median critical shear stress for d_{50} increases by 111%. Additionally, there is a severe expansion in the range of critical shear stresses, with about 10% of the distribution characterized by friction angles in excess of 90°, indicating virtual immobility of this percentage of the d_{50} sizes. Because these calculations employ friction angle and protrusion data derived from grains placed on bed surfaces, they provide conservative estimates of the stresses required to mobilize the actual stream-bed surface; grains composing the bed surface likely will be characterized by lower protrusions and higher friction angles (Buffington et al. 1992; Jiang and Haff 1993), resulting in critical shear stresses greater than those estimated here.

Grain mobility and susceptibility to scour may be further reduced by redd microtopography that introduces form drag that decreases bed shear stresses (i.e., lowers τ'). Bar-form

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Table 4. Effects of spawning activity on grain mobility: percent change in τ_{c50} relative to the unspawned value and percentage of bank-full shear stress represented by this change.

	Surface coarsening (eq. 3)		Surface coarsening and sorting combined (eq. 4)[a]		Redd form drag (eq. 5)	
	% change in τ_{c50}	% of bank-full τ	% change in τ_{c50}	% of bank-full τ	% change in τ_{c50}	% of bank-full τ
Kennedy Creek	36	23	111	na	17–62	11–40
Montana Creek	67	22	73	na	26–89	8–29

[a]Evaluated using median critical shear stresses of calculated stress distributions (Fig. 6). τ_c values calculated from eq. 4 may represent instantaneous turbulent sweeps and so may not be comparable to the time-averaged values of unspawned τ_{c50} and bank-full τ used here (Buffington et al. 1992).

resistance in gravel-bedded rivers varies with stage and bed morphology but may comprise 10–75% of the total channel roughness (Parker and Peterson 1980; Prestegaard 1983; Hey 1988). Nelson and Smith (1989) presented a theoretical model that describes the shear stress dissipated by flow over regularly spaced channel-spanning bedforms as

$$(5) \quad \tau_{bf} = \tau_g \frac{C_D}{2\kappa^2} \frac{H}{\lambda} \left(\ln \left(\frac{H}{z_0} \right) - 1 \right)^2$$

where τ_{bf} and τ_g are the shear stresses dissipated by bed-form and grain roughness, respectively, C_D is approximately equal to 0.21 for fully separated flow (Smith and McLean 1977), and H and λ are the bed-form amplitude and wavelength, respectively. Shear stress dissipated by redd topography decreases the basal shear stress available for sediment transport, effectively increasing the total shear stress required for grain mobility. The additional shear stress necessary to overcome redd form drag and initiate particle motion can be calculated from eq. 5, recognizing that at incipient motion $\tau_g = \tau_{c50}$ as defined by eq. 3.

Using τ_g equal to the postspawning τ_{c50} values calculated from eq. 3 together with $\lambda = 2.0$ m, $H = 0.10$–0.20 m, and $z_0 = 0.1 d_{84}$ (of postspawning gravels), eq. 5 predicts that at incipient grain motion, redd topography dissipates 2.1–7.8 Pa of channel shear stress in Kennedy Creek and 2.2–7.6 Pa in Montana Creek. In Kennedy Creek, these τ_{bf} values increase the critical shear stress of unspawned gravels (12.5 Pa) by 17–62% and constitute 11–40% of the bank-full shear stress (19.4 Pa). Similarly in Montana Creek, redd form drag increases the unspawned τ_{c50} value (8.5 Pa) by 26–89% and represents 8–29% of the bank-full shear stress (26.1 Pa). Our analyses indicate that the combined influence of redd form drag, surface coarsening, and sorting more than doubles τ_{c50} in Kennedy and Montana creeks (Table 4).

Spawning-related changes in packing of bed material, however, may also affect grain mobility. Redd construction loosens the stream bed, likely causing it to be less imbricated (i.e., have lower friction angles) and thus more easily mobilized (e.g., Church 1978; Reid et al. 1985; Li and Komar 1986). However, loose packing of the surface layer is unlikely to persist for long, as its deep intergranular pockets provide high friction angle locations that will trap fine material (e.g., Kirchner et al. 1990) that is normally in motion at flows less than critical (e.g., Jackson and Beschta 1982). These finer grains partially bury the larger loosely packed ones, likely decreasing their mobility by lowering grain protrusion and increasing friction angles (Buffington et al. 1992).

While we observed that redd gravels were typically more loosely packed than non-redd surfaces, the significance of bed loosening relative to that of surface coarsening and sorting is unknown. As we are unaware of any studies that have thoroughly quantified the influence of packing on grain mobility, we cannot estimate the importance of bed loosening by salmonids. Nevertheless, the influence of changes in packing on bed surface mobility would likely counteract the other spawning-related effects to some degree. Although critical shear stress calculations for Kennedy Creek indicated that postspawning bed mobility, and thus scour, should occur at stages significantly in excess of bank-full, field observations show that the postspawning bed surface mobilized during an approximately bank-full event. This bed mobility and scour at roughly the bank-full stage implies that loosening of the bed surface offsets approximately 32–44% of the increase in critical shear stress indicated by our calculations, implying that the net effect of spawning is reduced bed mobility, despite loosening of the bed surface.

Another issue remaining to be investigated is the longevity of salmonid modifications of the channel. Well-sorted bed surfaces tend to be hydraulically rough and have deep intergranular pockets that readily trap fine material. Salmonid-altered gravels may progressively decrease in size and become more poorly sorted in channels with high sediment supply that transport fine sediment at low to moderate stages. P.E. Porter (cited by Everest et al. 1987), for example, found that the percentage of fine particles (<1 mm) in stream-bed gravels of Montana Creek returned to prespawning levels within 1–2 months. Redd topography, however, may discourage fine sediment deposition above egg pockets by forcing both upstream deposition within redd pits and downstream suspension over tailspills (e.g., Everest et al. 1987). Nevertheless, persistence of redd forms is unlikely in streams that normally lack such microtopography as part of their bed morphology.

Implications

The close correspondence between egg burial depths and scour depths during typical annual high flows implies a finely tuned adaptation to long-term rates of sediment transport and implies high salmonid population sensitivity to variations in scour depths. The greater than 60% embryo survival for a bank-full flood inferred from Fig. 3 may define a reasonable minimum estimate of long-term mortality, as bank-full flows represent a predictable minimum annual peak discharge. The lower survival rate at higher discharges noted by Holtby and Healey (1986) may simply reflect scouring deeper into the population of embryos buried in the stream bed. Our data imply that changes in processes influencing scour depths could have dramatic impacts on salmonid population dynamics. Higher bedload transport rates resulting from increased sediment supply

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and consequent bed surface fining (Dietrich et al. 1989), for example, should directly increase scour depths. Many human activities (e.g., logging, road construction, urbanization) may increase bed scour by increasing the discharge and (or) the sediment supply to streams. Although numerous processes contribute to the ongoing decline of salmonid populations, changes in the depth of bed scour may be a key factor in the decline of certain species.

Our calculations indicate that spawning-related bed modifications may also influence scour and embryo survival by decreasing bed mobility. Bank-full flows for many rivers have recurrence intervals of roughly 1 or 2 years (Wolman and Leopold 1957; Wolman and Miller 1960; Dury 1973; Williams 1978). Hence, salmonid modifications that increase the critical shear stress may help protect embryos from scour during typical annual to biennial flows. The potential for spawning activity to reduce bed surface mobility may be particularly important at our study sites because of the coincidence of spawning timing and the highest monthly average discharges. Spawning activity in both channels may decrease bed mobility during periods when embryos are most susceptible to disruption and scour by high flows, implying that spawning alters the channel in a way that may enhance the probability of offspring survival. Spawning-related bed modifications will decrease as a mass-spawning salmonid population declines, potentially resulting in more frequent scour to egg-pocket depths, decreased embryo survival, and hence further population decline. Such feedback would make it increasingly difficult to reverse a declining population trend, especially if the decline coincided with changes in land use that lead to more frequent or higher discharges (e.g., Harr 1986; Booth 1991) or to bed surface fining and thus greater bed mobility (e.g., Dietrich et al. 1989) and scour. The influence of spawning-related bed modifications on scour and embryo survival warrants further investigation, however, because of the unquantified effect of bed loosening.

Fishing patterns that decrease fish size (e.g., Ricker et al. 1978; Ricker 1980) could also increase embryo mortality from bed scour. Smaller fish bury their eggs less deeply, leaving them more vulnerable to scour during bed-mobilizing events (e.g., van den Berghe and Gross 1984). Consequently, management practices that tend to reduce fish size might increase embryo mortality and would exacerbate any positive feedback between a declining population and bed mobility.

Examples of biological influences (other than the pervasive impact of human activity (Turner et al. 1990)) on physical environmental processes are relatively uncommon, and many ecological models assume that animals passively respond to environmental constraints. Our observations, however, suggest a potential feedback mechanism between spawning-related habitat modification, bed mobility, and embryo survival. This feedback is likely to be significant only for mass-spawning species using low-gradient gravel-bedded channels in which bed scour typically involves a fraction of the full thickness of the alluvium composing the stream bed. This mechanism is unlikely to significantly affect dispersed-spawning species inhabiting steep cobble and boulder-bedded channels in which frequent scour incorporates most of the available spawning gravel. In such channels the timing of spawning may represent a fundamental adaptation to channel-bed mobility (Montgomery 1994).

Acknowledgments

This research was supported by grants TFW FY92-010 and FY94-004 from the Washington State Timber–Fish–Wildlife agreement. T.P.Q. also acknowledges support of the H. Mason Keeler endowment and the U.S. Forest Service. We thank Anne Biklé and Kevin Schmidt for help with fieldwork. The manuscript was improved by an insightful discussion with Matt Kondolf and by comments from Rick Smith and two anonymous reviewers.

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Simulating winter flounder population dynamics using coupled individual-based young-of-the-year and age-structured adult models[1]

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Abstract: Population dynamics of winter flounder (*Pleuronectes americanus*) are simulated using a detailed individual-based model for young-of-the-year coupled to an age-structured matrix model for adults. When run alone, the young-of-the-year model is initiated with user-specified female spawners. When coupled to the adult model, multiple-year simulations are performed with young-of-the-year survivors inputted to the adult model and the adult model is used to generate the female spawners each year. Model predictions of young-of-the-year densities, growth rates, and mortality rates were within the range observed for the Niantic River population. Model predictions and Niantic River data both showed recruitment (survivors to age 1) to level off at high numbers of spawners. Predicted recruitment at high numbers of spawners was two to three times that for the same conditions under density independence. Analysis of a 200-year simulation indicated that recruitment was largely set by metamorphosis, with density-dependent growth and survival rates operating during the larval and juvenile life stages. The relationship of our modelling approach to previous approaches, the implications of our results to understanding winter flounder population dynamics, and possible further elaborations of the model are discussed.

Résumé : La dynamique des populations de sole (*Pleuronectes americanus*) est simulée par un modèle détaillé des comportements individuels de jeunes de l'année couplé à un modèle pour les adultes ayant la forme d'une matrice qui reprend une structure par âge. Lorsqu'il fonctionne seul, le modèle des jeunes de l'année est lancé à partir de données relatives à des génitrices définies par l'utilisateur. Lorsqu'il est utilisé de concert avec le modèle pour les adultes, on pratique des simulations portant sur plusieurs années à partir du taux de survie des jeunes de l'année qui alimente lui-même le modèle des adultes, lequel produit des génitrices chaque année pour alimenter le modèle des jeunes de l'année. Les prévisions fournies par ce modèle de la densité démographique des jeunes de l'année, des taux de croissance et des taux de mortalité se situent à l'intérieur de la plage de valeurs observée chez la population de la rivière Niantic. Les résultats obtenus par modélisation et ceux des relevés effectués sur cette population montrent que le recrutement (survivants jusqu'à l'âge de 1 an) plafonne lorsque le nombre des géniteurs devient élevé. Toutes autres conditions étant égales, le recrutement prévu à un nombre élevé de géniteurs était deux à trois fois celui prévu lorsqu'il n'y avait pas de lien avec la densité. L'analyse des résultats d'une simulation sur 200 ans montre que le recrutement dépend largement de la métamorphose en supposant que des taux de croissance et de survie jouent aux stades des larves et des juvéniles. Nous évaluons le lien entre notre approche et les approches précédentes et la signification de nos résultats pour la connaissance de la dynamique de population de la sole, et nous considérons certains perfectionnements que nous pourrions apporter à notre modèle.
[Traduit par la Rédaction]

Introduction

Modelling population dynamics of many marine fishes requires long-term (multigenerational) simulations. Recruitment is believed set in the 1st year of life for many marine fishes (Sissenwine 1984; Rothschild 1986; Houde 1987; Fogarty et al. 1991; Bradford 1992). However, 1-year simulations designed to predict recruitment may not be adequate to fully predict the effects of recruitment on a population. Implications of young-of-the-year (YOY) dynamics on the population may

Received February 21, 1995. Accepted November 20, 1995.
J12789

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not be fully realized until these YOY individuals mature some years later. Moreover, their effects may persist for the remainder of their reproductive life and possibly, via their offspring, into subsequent generations. Ages of maturity of >3 years and maximum ages of >10 years are common in fishes (Winemiller and Rose 1992).

Two different scales of temporal resolution are appropriate for long-term simulation of marine fish populations. Fine resolution is desired for the 1st year of life, during which recruitment is likely set and when subtle changes in vital rates and episodic events can greatly affect recruitment (Houde 1987, 1989*a*). Coarser resolution must be used for age-1 and older fish because fine-resolution data are usually lacking. Adults and older juveniles of many marine species leave nearshore nursery areas for sites further offshore where detailed study of their biology is difficult. Also, while laboratory study is reasonable for some aspects of the YOY life stages (although see de Lafontaine and Leggett 1987; MacKenzie et al. 1990), laboratory study of adult fish is problematic.

Our approach to modelling population dynamics of marine fish is to use a detailed, individual-based model for YOY, and to couple the YOY model with an age-structured matrix model for adults for long-term simulations. The YOY model follows individuals on a daily time step permitting the needed fine resolution for 1st-year dynamics. The YOY model is used to predict the number and mean length of age-1 survivors from eggs. YOY model predictions can be used alone to better understand factors affecting 1st-year growth and survival, or can be coupled to the coarser age-structured matrix model of adults for long-term simulations. The YOY and adult models are coupled by using predictions of age-1 survivors from the YOY model as input into the adult model, which then projects these YOY through the rest of their lifetime. Each year the adult model is used to compute the number and size distribution of spawners as input to the YOY model for the upcoming year.

We chose an individual-based approach operating on a daily time step to simulate YOY dynamics because individual differences, and events and processes operating on the scale of days, can be potentially important to predicting recruitment. Most marine fish die during their 1st year of life. The relatively few fish that survive may represent a nonrandom subset of the initial set of individuals with respect to their attributes (e.g., spawning date, parentage, egg size, growth rate). Subtle differences in body size and growth, mortality, and timing of ontogeny (Houde 1987; Chambers and Leggett 1992; Crowder et al. 1992) and episodic events operating on the order of days (Houde 1989*a*; Rose et al. 1993*a*) can affect the size distribution and magnitude of recruitment. A major drawback of using a detailed YOY model is its great demand for data. Typically, most available information is needed for model formulation and parameter estimation, thereby preventing rigorous model validation.

Because of the nature of the data available and the necessity of long-term simulations, we chose an age-structured matrix projection (Leslie) model for adult (age 1 and older) dynamics. Matrix projection models have been used extensively to simulate fish population dynamics (e.g., DeAngelis et al. 1977, 1980; O'Neill et al. 1981; Goodyear 1985; Schaaf et al. 1987; Saila et al. 1991). These models tend to be empirically based, relying on estimates of age-specific survival, maturity, and fecundity. A critical parameter in the Leslie model is the 1st-year survival rate, or equivalently, the number of individuals surviving from eggs to age 1. For Leslie models with a fixed 1st-year survival rate (i.e., density independent), the population either remains stationary, forever increases, or becomes extinct (DeAngelis et al. 1980). Two commonly used approaches to obtain stable population trajectories are to solve for the magnitude of 1st-year survival that produces stability or to specify a density-dependent relationship between the numbers of eggs and age-1 survivors (e.g., DeAngelis et al. 1980; O'Neill et al. 1981). With our coupled model approach, the detailed YOY model is used to generate the number of individuals surviving from eggs to age 1.

In this paper, we couple an individual-based YOY model with a matrix model in a population-based analysis of winter flounder (*Pleuronectes americanus*). The YOY and adult models are described first. Predictions of growth and mortality rates from the YOY model under typical (base-line) conditions are compared with observed values from long-term monitoring of winter flounder in the Niantic River estuary, Connecticut. YOY model predictions are further analyzed to determine which characteristics of individuals may be predictive of survival. We attempt to corroborate the YOY model by comparing model predictions with 11 years of spawner–recruit data from the Niantic River. The degree of density dependence underlying the generated spawner–recruit curve is quantified by comparing model simulations with and without density-dependent feedbacks. A long-term simulation using the coupled YOY and adult models is analyzed to compare the effects of temperature, food levels, and predation intensity on recruitment. We conclude with a discussion of the relationship of our modelling approach to previous approaches, the implications of our results to our understanding of winter flounder population dynamics, and possible further elaborations of the model.

Winter flounder and the Niantic River

Winter flounder is an important component of the nearshore and estuarine fauna in waters of the Atlantic coast of North America from the Gulf of St. Lawrence to Chesapeake Bay and is of commercial and recreational importance (Klein-MacPhee 1978). For much of their range, adult winter flounder reside for most of the year in coastal to offshore ocean waters and enter natal estuaries in the late fall to spawn the following winter (Kennedy and Steele 1971; Klein-MacPhee 1978). YOY and yearlings, which are more tolerant of higher temperatures, remain in the estuary while adult fish return to the deeper waters during the summer (McCracken 1963; Klein-MacPhee 1978; Van Guelpen and Davis 1979).

We designed the YOY and adult models to represent estuaries found in the northeastern United States. Much of the information used to configure the model is from the Niantic River, a shallow estuary of Long Island Sound, and the nearby Narragansett Bay, Rhode Island. Winter flounder in the Niantic River are well studied as a result of monitoring related to the operations of the Millstone Nuclear Power Station (Northeast Utilities Service Company 1987, 1992, 1994). The Niantic River system consists of a distinct embayment (river), with little freshwater input, connected by a channel to the open Niantic Bay, which is part of Long Island Sound. The sampling programs in the river and bay covered ≈50 km^2. The samples in the river were taken generally at depths <2 m and those in

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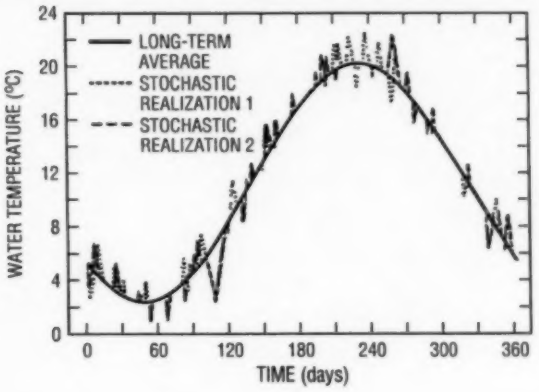
Fig. 1. Long-term average daily temperatures and two stochastic realizations of daily temperatures (°C) used in model simulations.



the bay at <8 m (Northeast Utilities Service Company 1992). Sampled data are representative of a shallow estuary because they are only minimally affected by the operations of the Millstone Nulcear Power Station. The power station is located on Long Island Sound, ≈2.0 km from the Niantic River mouth. The primary source of impact on winter flounder is larval entrainment, with an average of 9.0% of the river larvae becoming entrained annually (Northeast Utilities Service Company 1995); thermal discharges are confined to the immediate plant vicinity. Daily temperatures and prey information for the YOY model are from both the Niantic River and nearby Narragansett Bay. We use data from sampling stations in both the Niantic River and Niantic Bay for comparison with model predictions of winter flounder dynamics. As much as possible, data collected during 1983–1991 were used to compare with predictions of within year dynamics. Spawner–recruit data for the 1977–1987 year-classes were compared with predicted values.

Overall model description

The YOY and adult models represent population dynamics in a single, well-mixed 150 × 150 × 1.5 m deep compartment. The dimensions of the compartment are relevant only for the YOY model in determining winter flounder densities and how their consumption affects prey densities. The adult model tracks numbers of females in each age-class and is therefore unaffected by the dimensions of the compartment. The environmental conditions in the compartment consist of daily water temperature, the fraction of each day when there is light, and daily densities of a variety of prey types. All flounder and prey weights are reported as dry weight unless otherwise stated.

YOY model

The YOY model begins with the number and length distribution of spawning females and simulates the growth and mortality of their progeny as they develop through the egg, yolk-sac larval, larval, and juvenile life stages during their 1st year of life. Model simulations are for 1 year (365 days) beginning on January 1 (day = 1). The YOY model can be run either separately or coupled with the adult model. If run separately, the YOY model is initiated with a specified number and length distribution of female spawners and 1-year simulations are performed. When the models are coupled, the matrix model is used to generate the number and length distribution of female spawners each year, and 200-year simulations are performed.

Daily temperature and daylight

Daily temperatures (T, °C) for 365 days beginning on January 1 (day = 1) are generated as stochastic deviations from a regression equation fit to observed long-term average daily temperatures (Fig. 1). The long-term average was determined by fitting a trigonometric function to 13 years of mean daily temperatures recorded at the Millstone Nuclear Power Station in Niantic Bay, ≈2 km from the mouth of the Niantic River (Northeast Utilities Service Company 1992):

$$(1)\quad T = 11.37 - 5.99 \cdot \cos(0.0175 \cdot \text{day}) - 6.69 \cdot \sin(0.0175 \cdot \text{day})$$

where 0.0175 converts degrees to radians. Niantic River temperatures are slightly higher in the summer and lower in the winter than bay temperatures; absolute differences between temperatures at the mouth of the river and in the bay averaged 1.2°C for periods when both measurements were available. We use the temperatures for the bay because they are similar to temperatures experienced by winter flounder in northeastern U.S. estuaries and because the bay temperature record is complete.

Realizations of daily temperatures used in model simulations are simulated by randomly assigning successive runs of either positive (above the mean) deviations, negative (below the mean) deviations, or neutral (no deviations) from the mean temperature determined by eq. 1 (see Shuter et al. 1980; Rose and Cowan 1993). Maximum deviations in temperatures are generated from a gamma probability distribution with parameters estimated from the observed temperatures. The duration (days) of each deviation is then determined from the magnitude of the deviation on the basis of a regression fit to observed data. A neutral run, defined as observed temperatures being within 1°C of the long-term mean, can result after each positive or negative run. Daily temperatures are determined by linearly interpolating temperatures between the beginning and end of each positive or negative run such that the maximum deviation occurs at the midpoint of the run (Fig. 1).

The fraction of the 24-h day that is considered daylight (DL) varies with the day of year (Brock 1981):

$$(2)\quad \text{DL} = 7.639 \cdot \frac{\text{acos}(-0.869 \cdot t_2)}{24}$$

$$t_2 = \tan\left(\frac{\pi}{180} \cdot 23.45 \cdot \sin\left(\frac{2\pi(284 + \text{day})}{365}\right)\right)$$

Equation 2 is based on latitude 41°N. The arguments of the trigonometric functions in eq. 2 include conversions from degrees to radians.

Spawning

Lengths (L_f; millimetres) for each of the spawning females are randomly selected from a length distribution that is either user specified (YOY model alone) or generated by the adult model (multiple-year simulations). The weight of each female (W_f; grams wet weight) is determined from length as $W_f = 0.005\,94\,(L_f/10)^{3.226}$ (Northeast Utilities Service Company

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1987). Day of spawning is determined by assigning degree-days required for spawning to each female from a triangular distribution with minimum = 40, mode = 125, and maximum = 370 (Buckley et al. 1991). On the basis of daily temperatures, each female spawns on the 1st day that degree-days in the simulation exceed her degree-day requirement for spawning.

The number (N_e) and average weight (W_e; milligrams) of eggs per female are determined from female wet weight and temperature on day of spawning (T_s) (modified from Buckley et al. 1991):

$$(3)\quad N_e = 1493.7 \cdot W_f + 7240.2 \cdot T_s - 13.9 \cdot W_f \cdot T_s$$

$$(4)\quad W_e = 55.86 + 0.006 \cdot W_f - 3.82 \cdot T_s + 0.0016 \cdot W_f \cdot T_s$$

Eggs and yolk-sac larvae

Each female's eggs are followed as an individual cohort through the egg and yolk-sac larval stages. Timing of hatching and initiation of first feeding are determined by accumulating daily fractional development rates until the cumulation exceeds 1. Daily fractional development to hatching (DV_e) and first feeding (DV_y) are computed each day on the basis of temperature (R.C. Chambers, unpublished data):

$$(5)\quad DV_e = 0.0331 - 0.00316 \cdot T + 0.00109 \cdot T^2$$

$$(6)\quad DV_y = 0.174 - 0.02959 \cdot T + 0.00383 \cdot T^2$$

The length of larvae at 1st feeding (L_{ff}; millimetres) in each female cohort is determined from egg weight (W_e) (L. Buckley, National Marine Fisheries Service, Narragansett, R.I., personal communication):

$$(7)\quad L_{ff} = 3.518 + 0.01573 \cdot W_e$$

Weight (milligrams) is then determined from the length–weight relationship of larvae (see eq. 9).

The number of eggs and yolk-sac larvae in each female egg cohort is reduced daily according to the fraction dying. The daily mortality fraction of eggs (M_e) consists of the sum of a temperature-dependent component, based on a parabolic relationship between temperature and hatching success in the laboratory (Williams 1975), plus a constant fraction of 0.16:

$$(8)\quad M_e = (0.413 - 0.11315 \cdot T + 0.0084 \cdot T^2) + 0.16$$

The mortality fraction of 0.16 is similar to the mortality rate reported for eggs of Atlantic herring (*Clupea harengus*) (Dahlberg 1979), which, like winter flounder, have unguarded, demersal eggs. The fraction of yolk-sac larvae dying (M_y) is assumed to be 0.15 (instantaneous rate of 0.16 larvae/day), which is within the range for marine pelagic larvae in general (Dahlberg 1979; Bailey and Houde 1989).

Larvae and juveniles: initial numbers and sizes

Beginning at first feeding, individuals are followed day by day through growth and mortality processes. Initial lengths and weights of larvae depend only on female size and temperature at spawning; larvae from a given female are assigned the same initial weight and length.

The weight (milligrams) of each individual is converted into length (L; millimetres) daily on the basis of the following length–weight relationships (Frame 1972; Beyer and Laurence 1980):

$$(9)\quad L = \begin{cases} 8.148 \cdot W^{0.21} & \text{for larvae} \\ 10.723 \cdot W^{0.28} & \text{for juveniles} \end{cases}$$

Weight is partially uncoupled from length; length is allowed only to increase whereas weight is allowed to increase or decrease. On days that individuals lose weight their lengths are not changed. Following a weight loss, lengths increase only after the individual has recovered to the weight predicted by the length–weight relationship. Larvae develop into juveniles when their length exceeds 8 mm (Chambers and Leggett 1987).

Larvae and juveniles: growth

The weight (W_t; milligrams) of an individual fish is incremented daily beginning with first feeding by means of a bioenergetics equation:

$$(10)\quad W_t = W_{t-1} + p \cdot C_{max} \cdot A - R_{tot}$$

where C_{max} is the maximum consumption rate (milligrams per day), p is the proportion of C_{max} realized, A is the assimilation efficiency, and R_{tot} is the total metabolic rate (milligrams per day). The value of p depends on the number of each prey type consumed and is described below in the section on the computation of p.

C_{max} depends on an individual's weight and the water temperature:

$$(11)\quad C_{max} = \begin{cases} 0.431 \cdot W^{0.81} \cdot F(T) & \text{for larvae} \\ 0.65 \cdot W^{0.78} \cdot F(T) & \text{for juveniles} \end{cases}$$

$$(12)\quad F(T) = \left(\frac{K1 \cdot e^{G1(T-\theta)}}{1.0 + K1 \cdot (e^{G1(T-\theta)} - 1.0)}\right) \cdot \left(\frac{K4 \cdot e^{G2(TL-T)}}{1.0 + K4 \cdot (e^{G2(TL-T)} - 1.0)}\right)$$

$$G1 = \left(\frac{1.0}{T0 - \theta}\right) \ln\left(\frac{0.98 \cdot (1.0 - K1)}{0.02 \cdot K1}\right)$$

$$G2 = \left(\frac{1.0}{TL - TM}\right) \ln\left(\frac{0.98 \cdot (1.0 - K4)}{0.02 \cdot K4}\right)$$

C_{max} as a function of weight is from laboratory data under excess prey levels (Beyer and Laurence 1980; R. Wright, Ohio State University, Columbus, Ohio, personal communication). The function relating C_{max} to temperature, $F(T)$, rises rapidly, reaches one at an optimal temperature, and then drops slowly to zero at a maximum temperature (Hewitt and Johnson 1987). Six parameters are required to specify the function (Table 1). A is assumed constant at 0.7 (Brett and Groves 1979; Beyer and Laurence 1980).

Metabolic losses (R_{tot}; milligrams per day) are determined as a routine component (R_r), which depends on weight and temperature, and an active component, which we represent by a multiplier (2.5) of routine metabolism for DL:

$$(13)\quad R_{tot} = R_r + (2.5–1.0) \cdot R_r \cdot DL$$

An activity multiplier of 2.5 is typical for marine larvae (Rombough 1988) and was used in a previous larval winter flounder bioenergetics model by Beyer and Laurence (1980).

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Table 1. Parameter values for the temperature dependence of maximum consumption (eq. 12).

Parameter	Definition	Value	
		Larvae	Juveniles
Θ	Temperature at which rate is K1 times the maximum rate	0	3
TL	Temperature at which rate is K4 times the maximum rate	25	30
T0	Temperature on the ascending portion at which rate is 0.98 of the maximum rate (<TM)	8	14
TM	Temperature on the descending portion at which the rate is 0.98 of the maximum rate	14	18
K1	Adjustment to rate associated with temperature Θ	0.30	0.25
K4	Adjustment to rate associated with temperature TL	0.01	0.01

Note: Values are based on typical temperatures at the time that winter flounder larvae and juveniles are present in the Niantic River (Northeast Utilities Service Company 1992), and reported laboratory and field temperature preferences of winter flounder (see Klein-MacPhee 1978).

Table 2. Weights, lengths, and specified turnover rates of each of the zooplankton and benthic prey groups.

Prey type	Weight (mg)	Length (mm)	Turnover rate (day^{-1})
Tintinnids[a]	0.000 011 6	0.05	0.33
Rotifers[b]	0.000 16	0.2	0.3
Invertebrate larvae[c,d]	0.001 3	0.35	0.2
Copepod nauplii[e]	0.000 2	0.2	0.2
Harpacticoid copepods[f]	0.001 2	0.5	0.15
Nematodes[g]	0.002 8	1.0	0.15
Ostracods[h]	0.003	1.0	0.15
Amphipods[h]	0.005 2	2.0	0.1
Amphipods[h]	0.068	4.0	0.1
Polychaetes[h]	0.001 7	3.5	0.05
Polychaetes[h]	0.003 2	4.0	0.05
Polychaetes[h]	0.005 7	4.5	0.05
Polychaetes[h]	0.009 5	5.0	0.05
Polychaetes[h]	0.015 1	5.5	0.05
Polychaetes[h]	0.023 0	6.0	0.05
Polychaetes[h]	0.033 9	6.5	0.05
Polychaetes[h]	0.048 5	7.0	0.05
Polychaetes[h]	0.067 7	7.5	0.05
Polychaetes[h]	0.092 5	8.0	0.05
Polychaetes[h]	0.123 9	8.5	0.05
Polychaetes[h]	0.164 3	9.0	0.05
Polychaetes[h]	0.212 2	9.5	0.05
Polychaetes[h]	0.272 0	10.0	0.05
Polychaetes[h]	0.385 0	10.75	0.05
Polychaetes[h]	0.534 6	11.5	0.05
Polychaetes[h]	0.725 6	12.25	0.05
Polychaetes[h]	0.967 2	13.0	0.05
Polychaetes[h]	1.268 5	13.75	0.05
Polychaetes[h]	1.634 0	14.5	0.05

Note: Weights and lengths are assigned to prey groups using published length–weight relationships, as follows.

[a]Verity (1987).

[b]G. Klein-MacPhee, unpublished data.

[c]Marine Research, Inc. (1980).

[d]Day and McEdward (1984).

[e]Durbin and Durbin (1981).

[f]Dumont et al. (1975).

[g]Combination of a width to weight relationship (Holland et al. 1989), a specific weight of 1.1 (Teitjen 1969), and a volume to length and width relationship (Warnick and Price 1979).

[h]Holland et al. (1989).

Routine metabolism is determined as a function of larval weight and a Q_{10} temperature relationship:

$$R_r = \begin{cases} 0.107 \cdot W^{0.74} \cdot G(T) & \text{for larvae} \\ 0.0675 \cdot W^{0.792} \cdot G(T) & \text{for juveniles} \end{cases} \quad (14)$$

$$G(T) = e^{\frac{\log_e(Q_{10})}{10.0}(T-T_r)} \quad (15)$$

where T_r is the temperature at which the weight-dependent routine metabolic rate occurs. The equation for routine metabolism as a function of weight was combined from Beyer and Laurence (1980) for larvae and Voyer and Morrison (1971) for juveniles. On the basis of the temperatures at which metabolic rates were reported, T_r is set to 8°C for larvae and 15°C for juveniles. Q_{10} is set to 1.9 for larvae and juveniles, as a compromise among reported values. Q_{10} values of larval and early juvenile winter flounder vary from about 2.0 to 3.5 (Laurence 1975). Rombough (1988) reported a range of Q_{10} values of 1.5–4.9 for larvae; Houde (1989*b*) reported a Q_{10} value of 1.5 derived from a cross-species analysis of metabolic rates of marine fish larvae.

We adjusted weight loss predicted from the bioenergetics equation so that individuals starve after a set number of days if no feeding occurs. Larval weight loss is set so that they would lose 35% of their current weight (Letcher et al. 1996) after 3 days under no-food conditions ($p = 0$ in eq. 10). The point of no return and time to 50% mortality for starved winter flounder larvae are ≈5–7 days (Beyer and Laurence 1980; Buckley 1980; McGurk 1984). We use a value of 3 days to reflect the finding that previously fed larvae starved faster than never-fed larvae (Miller et al. 1988). Juveniles are restricted to lose no more than 2% of their body weight in a day.

Computation of *p*

The fraction of maximum consumption realized, p, is based on random encounters of individual larvae or juveniles with different prey groups. p is computed as

$$C_r = \sum_{i=1}^{n} B_i \mathrm{PW}_i \quad (16)$$

$$p = \frac{C_r}{C_{max}} \quad (17)$$

where C_r is the biomass (milligrams) of prey consumed, B_i is the number of prey group i eaten, and PW_i is the weight (milligrams) of an individual of prey group i. B_i is generated for each

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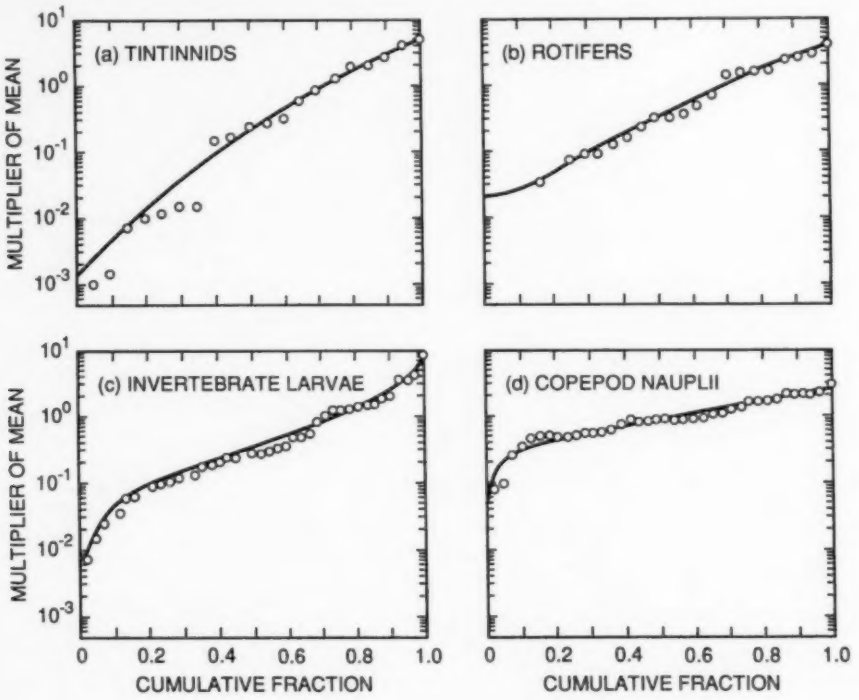
Fig. 2. Cumulative distribution functions of each of the four zooplankton prey groups relating the multiplier of the mean density to the cumulative fraction of samples with values less than some value.



prey group and individual as a random deviate from a binomial distribution, dependent on the realized number of encounters (E_i) and probability of capture (PC_i). The summation over i prey groups in computing C_r is performed in order of decreasing preference of prey groups (see the section below on prey selection). p is bounded between 0 and 1 because $0 \leq C_r \leq C_{max}$.

Prey

Prey types

Four zooplankton and 25 benthic prey groups are represented. Zooplankton prey groups correspond to tintinnids, rotifers, invertebrate larvae, and copepod nauplii, which comprise most of the zooplankton in the diet of larval winter flounder (Pearcy 1962*a*; Marine Research, Inc. 1980; G. Klein-MacPhee, unpublished data). Benthic prey groups consist of a series of 25 weight classes with each weight class assumed to represent either harpacticoid copepods, nematodes, ostracods, amphipods, or polychaetes (Table 2). These prey groups commonly comprise most of the prey found in the diet of juvenile winter flounder (Pearcy 1962*a*; Mulkana 1966). Each prey group is defined by an average weight (PW_i), length (PL_i; millimetres), and specified turnover rate (PT_i; number per day), which do not change over time (Table 2), and time-dependent equilibrium densities (PD^e; see section below on temporal dynamics of prey).

Prey encounters

The realized number of encounters of each prey type (E_i) is generated from the mean encounter rate E_i^M. For larvae, the realized number of encounters with zooplankton is obtained from E_i^M using a cumulative distribution function (CDF). The CDF is computed as the cumulative fraction of total samples whose observed zooplankton densities are less than a specified density. Dividing the zooplankton densities by their mean density results in a multiplier of the mean on the y axis and the cumulative fraction of samples on the x axis (Fig. 2). A random number between 0 and 1 is generated and the CDF is used to obtain the multiplier of the mean. The product of the mean encounter rate and the multiplier of the mean yields the realized encounter rate. For benthos, the realized number of encounters is generated as a deviate from a Poisson distribution with the parameter of the Poisson equal to the mean encounter rate.

E_i^M is calculated as the product of search volume (or area) and prey density:

$$(18) \quad E_i^M = S_i \cdot PD_i$$

For larvae, which feed in the water column, S_i is the volume (litres) searched for prey and PD_i is the density of the zooplankton prey group (number per litre). For juveniles, who reside and feed on the bottom, S_i is the area searched for prey (square metres) and PD_i is the density of benthic prey group i (number per square metre). Search volume or area is specific to each prey group because it depends on prey size. S_i for larvae is computed as the volume of a cylinder with a radius equal to the reactive distance (RD_i) and a height equal to the distance swum during DL:

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$$(19)\quad S_i = \pi \cdot (\mathrm{RD}_i \cdot F_{\mathrm{RD}})^2 \cdot (a_{\mathrm{larv}} \cdot \mathrm{L} \cdot \mathrm{DL} \cdot 3600.0 \cdot 24.0) \cdot 10^{-6}$$

where F_{RD} is the reduction in RD owing to turbidity, a_{larv} is the swimming speed of larvae in body lengths (BL) per second, and multiplying by 10^{-6} converts values in cubic millimetres to litres. Search area for juveniles (S_i) is the area of the rectangle formed by twice the RD and the distance swum during DL:

$$(20)\quad S_i = 2.0 \cdot \mathrm{RD}_i \cdot F_{\mathrm{RD}} \cdot (a_{\mathrm{juv}} \cdot L \cdot \mathrm{DL} \cdot 3600.0 \cdot 24.0) \cdot 10^{-6}$$

where a_{juv} is the swimming speed (BL per second) of juveniles and mutiplying by 10^{-6} converts values in square millimetres to square metres. RD is based on the angle of acuity of the individual (α) and the height (assumed length) of the prey item:

$$(21)\quad \mathrm{RD}_i = \frac{\mathrm{PL}_i}{2.0 \cdot \tan\left(\frac{\alpha}{2.0}\right)}$$

α decreases with increasing fish length (Breck and Gitter 1983):

$$(22)\quad \alpha = 0.0167 \cdot e^{9.14 - 2.4\ln(L) + 0.229\ln(L)^2}$$

Equations 21 and 22 result in larger flounder having a greater RD for a given size of prey, and for a flounder of a given length, larger prey are detected at a greater distance than smaller prey.

A turbidity factor F_{RD} reduces the RDs predicted by eqs. 21 and 22. Equations 21 and 22 were determined in laboratory experiments with low-turbidity water. We reduce RDs in proportion to their value to reflect the fact that the effect of turbidity increases with increasing RD (Vinyard and O'Brien 1976; Chesney 1989):

$$(23)\quad F_{\mathrm{RD}} = \begin{cases} 0.8 - 0.025 \cdot \mathrm{RD} & \text{if RD} < 15.0 \\ 0.4 & \text{if RD} \geq 15.0 \end{cases}$$

F_{RD} acts to reduce the RDs from 80% (for RD = 1 mm) to 40% (for RD > 15 mm) of the values predicted by eqs. 21 and 22. Vinyard and O'Brien (1976) reported that RDs in turbid waters similar to those in Long Island Sound near the Niantic River (≈30 mg · L^{-1} or JTU) were 50–25% of RDs determined in clear water.

We used an a_{larv} of 1.5 BL/s and an a_{juv} of 0.3 BL/s. Larval swimming speeds generally vary from 1.0 to 3.0 BL/s (Blaxter 1986); mean sustained cruising speeds of adult fish are typically 0.2–0.5 BL/s (Beamish 1978).

Prey capture

We use a single-capture probability term and a prey selection algorithm to encompass all of the steps from encounter to successful ingestion. Capture probabilities by larvae (PC_i) are specified for each of the zooplankton prey groups and depend on the size of the flounder:

$$(24)\quad \mathrm{PC}_i = \begin{cases} 0.5 + 0.0375 \cdot L & \text{for tintinnids, rotifers, and invertebrate larvae} \\ 0.0398 \cdot W^{0.2} & \text{for copepod nauplii} \end{cases}$$

Probability of capture of nauplii is from Beyer and Laurence (1980); probability of capture for the remaining zooplankton groups is based on general patterns of capture success observed for marine fish larvae (Blaxter 1986). For a larva of average weight for its length, capture probabilities of nauplii are less than for the other zooplankton prey groups.

PC_i values for benthic prey by juveniles increase with decreases in the ratio of prey length to flounder length (PL_i/L):

$$(25)\quad \mathrm{PC}_i = \begin{cases} 0.5 - 1.75 \cdot (\mathrm{PL}_i/L) & \text{for } \mathrm{PL}_i/L \leq 0.2 \\ 0.0 & \text{otherwise} \end{cases}$$

Prey of lengths greater than 20% of flounder length are assumed to be too large to eat. Gape size in winter flounder increases from about 4 to 10 mm as length increases from 50 to 200 mm (M. Armstrong, University of New Hampshire, Durham, N.H., personal communication). This corresponds to flounder eating prey with maximum sizes that are 5–10% of their length. Diet analysis of winter flounder from two Rhode Island estuaries showed that larger winter flounder (range 10–80 mm) ate progressively larger prey items and that most prey eaten had lengths less than 10–30% of the flounder length (Mulkana 1966).

Prey selection

Prey selection by larvae is based on principles of optimal foraging (Krebs 1978; Hughes 1980; Hart 1986). The optimal foraging algorithm we used permits larvae to consume more preferred prey groups when they are abundant and switch to less preferred prey groups when the more preferred ones are rare. To determine which zooplankton prey groups are candidates to be eaten by a larva on a given day, the prey are first ranked in descending order of preference. Preference of prey is equated with prey weight multiplied by capture probability ($PW_i \cdot PC_i$). Handling times are assumed equal for all prey groups and arbitrarily set to 1 s. Prey are then added to the diet (the summation in eq. 16) in decreasing order of preference. Prey groups are included in the diet until the consumption rate index with the added prey group i (Q_i) decreases compared with the index with prey groups 1 through i - 1 (Q_{i-1}). The consumption rate index is

$$(26)\quad Q_i = \frac{\sum_{j=1}^{i} \mathrm{PW}_j B_j T_{\mathrm{D}}}{1.0 + \sum_{j=1}^{i} H_j B_j T_{\mathrm{D}}}$$

where Q_i is the consumption rate index corresponding to eating from the most preferred prey group ($j = 1$) to the ith prey group, and T_D is the inverse of the approximate number of seconds during daylight hours (2.3×10^{-5}). Individuals are assumed to consume all individuals of each prey group, starting from the most preferred, until all eligible prey groups have been consumed or C_r exceeds C_{max}. Depending on relative encounter rates and capture successes, larvae may or may not select prey groups in order of prey size.

Juveniles are assumed to prefer benthic prey in order of largest to smallest prey lengths. To determine which benthic prey groups are candidates to be eaten by juveniles, prey groups are ranked in decreasing order of their size. Probability

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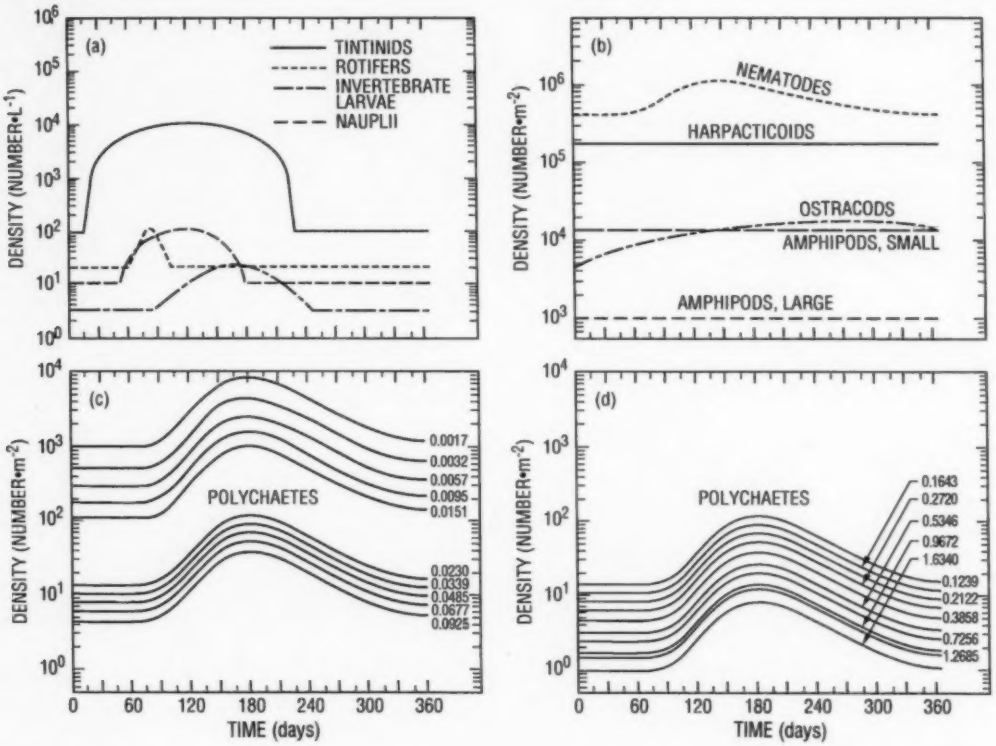
Fig. 3. Equilibrium densities over time for each prey group assumed in model simulations: (*a*) zooplankton and (*b–d*) benthos. The functions relating equilibrium densities to day of year are based on observed zooplankton densities from several studies of Narragansett Bay (Verity 1987; Durbin and Durbin 1988; G. Klein-MacPhee, unpublished data) and observed benthos densities in the Niantic River (Tietjen 1969) and Narragansett Bay (Rudnick et al. 1985; Frithsen 1989). Each polychaete prey type in *c* and *d* is denoted by its value of weight per individual (see Table 2).



of capture restricts potential prey groups eaten by juvenile flounder to lengths less than 0.2 of flounder lengths (eq. 25). As with larvae, juveniles are assumed to consume all individuals of each prey group, in accord with prey group rankings, until all eligible prey groups have been consumed or C_r exceeds C_{max}.

Temporal dynamics of prey

Densities of each prey group (number per litre for zooplankton and number per square metre for benthos) are updated daily on the basis of a modified logistic growth equation and the total number of prey group *i* consumed ($EAT_{i,t}$). The logistic formulation uses equilibrium densities ($PD^e_{i,t}$) and a specified PT_i (day^{-1}) to simulate prey population growth:

$$(27)\quad PD_{i,t+1} = PD_{i,t} + 2.0 \cdot PT_i \cdot \left(1.0 - \frac{PD_{i,t}}{PD^e_{i,t}}\right) \cdot PD_{i,t} - EAT_{i,t}$$

The multiplier 2.0 in eq. 27 allows PT_i to be realized when simulated densities are at one-half equilibrium densities. In the usual logistic formulation (without the multiplier 2.0), PT_i values are realized in the limit as population density approaches zero.

Equilibrium densities for each zooplankton and benthic prey group follow general seasonal patterns for the year based on empirical data from the Niantic River and Narragansett Bay (Fig. 3). Zooplankton data, except for tintinnids, are from two studies of microzooplankton in Narragansett Bay. One study consists of samples taken approximately weekly from mid-March through April in 1986 at three stations (G. Klein-MacPhee, unpublished data). The other study consists of weekly samples at two stations from March through June 1976 (Durbin and Durbin 1981, 1988). Estimates of tintinnid equilibrium densities are based on weekly samples for 1980–1982 at one station in Narragansett Bay (Verity 1987). Benthic data used to estimate equilibrium density functions consist of monthly collections of harpacticoid copepods, nematodes, ostracods, amphipods, and polychaetes during 1965 at two stations in the Niantic River (Tietjen 1969) and general seasonal patterns observed in Narragansett Bay (Rudnick et al. 1985; Frithsen 1989). Prey densities reported by Teitjen (1969) for harpacticoid copepods, nematodes, and ostracods are assigned to 1 weight class, for amphipods to 2 weight classes, and for polychaetes to 20 weight classes (Table 2). For amphipods and polychaetes, densities are split among weight classes by assuming equal biomass in all weight classes.

PT_i values are assumed constant over the year for each prey group (Table 2). PT_i values should be similar to, or exceed,

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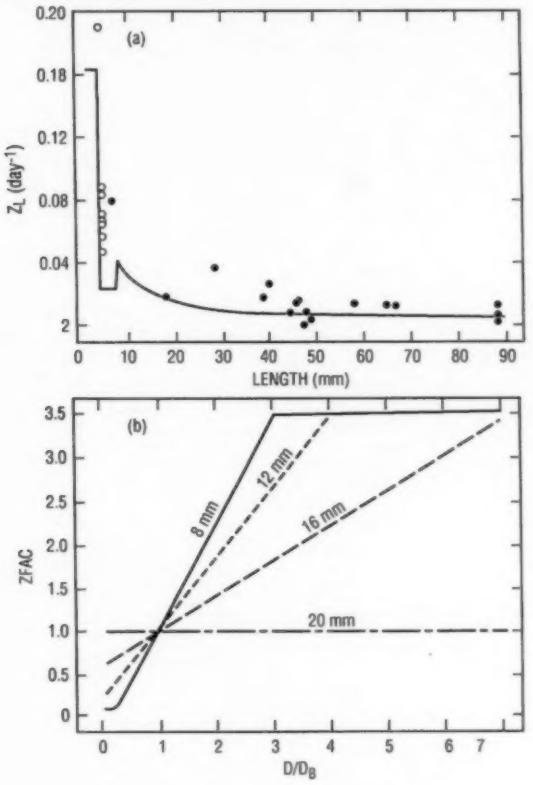
Fig. 4. (*a*) Length-based mortality rate and (*b*) the density-dependent modifier for juvenile winter flounder 8–20 mm in length (eqs. 31 and 32). Predation mortality is the product of the length-based mortality rate (Z_L) and the density-dependent modifier (ZFAC). Z_L is assumed constant for larvae and related to length for juveniles. ZFAC varies around one depending on the ratio of simulated juvenile density (D) to a specified base-line juvenile density (D_B). Observed rates for the length-based mortality component (●,○) are from data reported in Northeast Utilities Service Company (1992) and Pearcy (1962*b*); open circles are mortality rates from hatching to metamorphosis. ZFAC is based on data reported in van der Veer (1986); D_B is set to 0.1·m^{-2} on the basis of typical early stage juvenile densities in the Niantic River (Northeast Utilities Service Company 1992).



observed rates because observed rates occurred with densities near equilibrium values. Realized turnover rates in model simulations will be less than specified rates for prey densities greater than one-half equilibrium densities. PT_i values of zooplankton range from 0.2 to 0.33·day^{-1}. Turnover rates of marine copepods in general range from 0.044 to 0.25·day^{-1} for temperatures similar to those experienced by winter flounder larvae (3–15°C) (Huntley and Lopez 1992). Turnover rates in Narragansett Bay were 0.02–0.1·day^{-1} for nauplii at 4°C, and 0.1–0.6·day^{-1} for *Acartia* spp. from March to June (Durbin and Durbin 1981). Specified turnover of benthos in the model ranges from 0.05 to 0.15·day^{-1}. Dividing reported Narragansett Bay annual production to biomass ratios for harpacticoid copepods, nematodes, and ostracods by a 90-day growing season (Rudnick et al. 1985) results in turnover rates of 0.04-0.2·day^{-1}.

Densities of zooplankton and benthos prey groups are potentially reduced by winter flounder consumption. The total number of prey type i consumed (per litre or square metre) each day ($EAT_{i,t}$) is

$$(28)\quad EAT_{i,t} = \sum_{j=1}^{N} \frac{B_{j,i}\, m_j}{V \cdot 10^4}$$

where $B_{j,i}$ is the number of prey type i eaten by individual j, N is the number of model individuals, m_j is the number of population individuals represented by individual j (see section on numerical considerations below), and V is the size of the simulated compartment (litres for larvae and square metres for juveniles).

Prey availability

To produce realistic growth in juvenile winter flounder in model simulations, benthic prey densities were adjusted for availability to winter flounder by multiplying by the constants of 0.0002 for harpacticoid copepods, 0.000 17 for nematodes and ostracods, and 0.000 01 for amphipods and polychaetes. These values for the availability factors were determined by trial and error model simulations until the predicted mean lengths of juveniles over time were within the range observed in the Niantic River. Availability factors are needed because despite high densities, much of the benthos is not vulnerable to winter flounder predation. Miron et al. (1991) observed that the polychaete *Nereis* was mostly active during the night and estimated that it was outside of its burrow <0.2% of a 24-h day. Hall et al. (1990) concluded that benthic prey are generally unavailable to fish, as dab (*Limanda limanda*) in cages fed poorly despite the presence of prey.

Larvae and juveniles: mortality

Mortalities resulting from starvation and predation are represented. Starvation mortality depends on an individual's weight, whereas predation mortality depends on an individual's stage or length and, for newly metamorphosed juveniles, their density.

An individual dies in the simulations if its weight becomes less than a minimum sustainable weight (W_{min}). W_{min} is defined as a fraction (K_{min}) of their expected weight based on their length (W_L):

$$(29)\quad W_{min} = K_{min} \cdot W_L$$

where W_L is the predicted weight from length (eq. 9). K_{min} is set to 0.65 for larvae and 0.5 for juveniles on the basis of general results for other species (Johnson and Evans 1991; Letcher et al. 1996).

Predation mortality (Z_T, number per day) is assumed to be a constant for larvae and a function of length and density for juveniles. Z_T is expressed as a product of a length-based rate and a density-dependent modifier:

$$(30)\quad Z_T = Z_L \cdot ZFAC$$

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$$(31)\quad Z_L = \begin{cases} 0.025 & \text{for larvae} \\ 0.6 / L^{1.1012} & \text{for juveniles} \end{cases}$$

$$(32)\quad \mathrm{ZFAC} = \begin{cases} 1.0 & \text{for larvae and for juveniles} > 20 \text{ mm} \\ -1.0 + 2.0 \cdot D_t / D_b + 0.1 \cdot L - 0.1 \cdot D_t / D_b \cdot L & \text{for juveniles} < 20 \text{ mm} \end{cases}$$

where Z_L is the length-based mortality rate (number per day), ZFAC is the density-dependent modifier to length-based mortality, D_t is the simulated density of juveniles (number per square metre) on day t, and D_b is the specified juvenile density (number per square metre) at which total mortality equals the length-based mortality (i.e., ZFAC = 1.0). The probability of dying ($P_D = 1 - e^{-Z_T}$) is evaluated daily for each individual on the basis of its length. The individual is assumed to have died if a generated random number from a uniform distribution between 0 and 1 is less than P_D.

The mortality rate of larvae is implicitly density dependent and the length-based rate is set to $0.025 \cdot \mathrm{day}^{-1}$ to obtain average survival rates from hatching to metamorphosis of 5.0–10.0%, similar to the 2.0–17.0% (mean of 8.0%) observed over 8 years in the Niantic River (Northeast Utilities Service Company 1992). Yolk-sac and early stage larvae exhibit higher mortality rates than late-stage larvae (Pearcy 1962*b*; Northeast Utilities Service Company 1992). Yolk-sac larval mortality rate was set to 0.15 (see section above on eggs and yolk-sac larvae). Combining daily the mortality fraction (0.15) and duration (9 days) yields a yolk-sac stage survival rate of about 25.0% (0.85^9); thus, to obtain ≈7.0% survival with a typical larval stage duration of 50 days implies a larval mortality rate of $0.025 \cdot \mathrm{day}^{-1}$. Larval mortality becomes density dependent when the winter flounder consumption rate exceeds the specified turnover rate of the zooplankton prey types, resulting in zooplankton densities dropping below equilibrium values. Reduced zooplankton densities cause slower growth and prolong the time individuals spend in the high-mortality larval stage.

The mortality rate of juveniles is explicitly density dependent for 8- to 20-mm juveniles and implicitly density dependent for juveniles >20 mm in length. The length-based component relates mortality rate to flounder length (Fig. 4*a*) and is based on reported mortality rates of larvae in the Niantic River (Northeast Utilities Service Company 1992) and juveniles in the Mystic River, Comnnecticut (Pearcy 1962*b*). For juveniles >20 mm, mortality becomes density dependent if consumption exceeds benthic prey turnover rates, resulting in a slower growth and, because mortality rate decreases with length, higher mortality. For juveniles <20 mm, the density-dependent component (ZFAC) increases or decreases the mortality rate predicted by the length-based component depending on juvenile density (Fig. 4*b*). The density at which ZFAC equals one (D_B) is set to $0.1 \cdot \mathrm{m}^{-2}$, a typical early stage juvenile density in the Niantic River (Northeast Utilities Service Company 1992). Equation 31 exhibits similar variation in mortality rates as a function of density (approximately fourfold for a threefold change in density) as observed for juvenile European plaice (*Plueronectes platessa*) <15 mm in the Dutch Wadden Sea (van der Veer 1986). Prey and predator densities underlying the density-dependent mortality relation are assumed to be similar between the Wadden Sea and Niantic River. Plaice densities in the Wadden Sea (van der Veer 1986) and juvenile winter flounder in the Niantic River (Northeast Utilities Service Company 1992) both show spring densities of $\approx 0.5 \cdot \mathrm{m}^{-2}$. Densities of large or adult caridean shrimp (*Crangon* spp.), dominant predators on newly metamorphosed flatfish, also range from near zero in the winter to $5.0–20.0 \cdot \mathrm{m}^{-2}$ during the spring and summer in the Wadden Sea (van der Veer and Bergman 1987) and in two estuaries near the Niantic River (Mystic River, Modlin 1980; Narragansett Bay, Frithsen 1989). Laboratory experiments show that winter flounder longer than 20 mm are not vulnerable to predation by seven-spine bay shrimp (*Crangon septemspinosa*) (Witting and Able 1993).

Adult model: closing the life cycle

The adult model closes the life cycle by projecting the number and mean length of females in each of 15 age-classes through time on an annual time step by using age-specific survivorship, fraction mature, and a von Bertalanffy growth curve. Each year the adult model promotes the numbers and mean lengths of female flounders in each age-class one year, receives the number of age-1 females and their mean length from the YOY model, and generates the numbers and size distribution of females spawners as input to the YOY model for the next year. Denote the number of females in each age-class i at time t as $n_i(t)$ and their mean lengths as $L_i(t)$, $i = 1–15$. For each year t, the YOY model generates the number, $n_1(t)$, and mean length, $L_1(t)$, of age-1 females. The number of females is one half the number of survivors from the YOY model (assuming a 50:50 sex ratio). The adult model then decrements the numbers of females in subsequent age-classes as

$$(33)\quad n_{i+1}(t+1) = s_i \cdot n_i(t)$$

where s_i is the assumed survival rate from age i to $i + 1$.

Mean length is incremented with age using a von Bertalanffy growth curve. The von Bertalanffy predicted age of a fish (x_i) with mean length L_i is first determined:

$$(34)\quad x_i = 0.35 - \frac{\log_e\left(1.0 - \dfrac{L_i(t)}{453.0}\right)}{0.3}$$

One year is added to the von Bertalanffy predicted age to determine mean length at age $i + 1$:

$$(35)\quad L_{i+1} = 453.0 \cdot (1.0 - e^{0.3 \cdot ((x_i + 1.0) - 0.35)})$$

Equation 35 is estimated from female length at age data for Niantic River winter flounder for 1977–1983 (Northeast Utilities Service Company 1987). We add 25 mm to the predicted mean lengths of age-1 survivors (≈55 mm) to be consistent with the predicted age-1 lengths of the von Bertalanffy curve (80 mm) so that lengths at subsequent ages would be correct. The von Bertalanffy curve was fit to individuals of age 1 year during the next spawning season and was not specifically designed to predict age-1 mean lengths.

Our method of incrementing the von Bertalanffy growth curve by 1 year to simulate growth of adults mimics the pattern of decreasing coefficients of variation (CVs) of adult lengths

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Fig. 5. Densities of winter flounder larvae (*a*) for the three base-line simulations and (*b*) observed in the Niantic River. Observed densities for each year for 1983–1989 and 1991 were fitted with Gompertz functions (see Northeast Utilities Service Company 1992 for details).

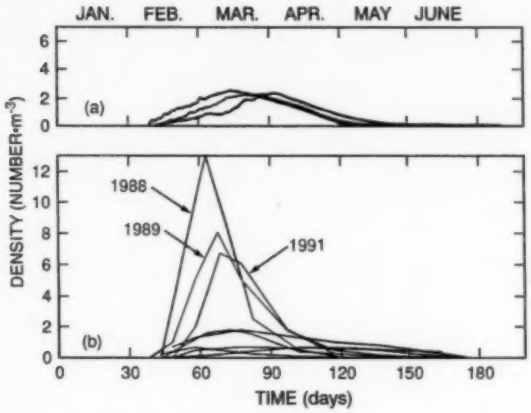
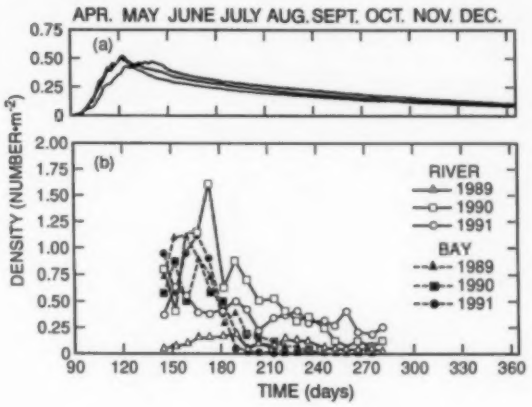


Fig. 6. Densities of winter flounder juveniles (*a*) for the three base-line simulations and (*b*) observed in the Niantic River and Bay. Observed densities are weekly mean densities at a river and a bay sampling station for 1989–1991 (see Northeast Utilities Service Company 1992 for details).



with age. Differences in mean lengths of age-1 individuals between year-classes are maintained throughout their lifetime. However, because the slope of the von Bertalanffy curve decreases with age, these differences decrease in magnitude with age. For example, age-1 cohorts with mean lengths of 38 versus 62 mm (difference of 26 mm) would differ by 19 mm at age 2, 8 mm at age 5, and <2 mm by age 9. CVs of length decrease from 28.0% for age-1 flounder, to 15.0% at age 2, to ≤10.0% for age-3 and older flounder in the Niantic River (Northeast Utilities Service Company 1987). Whether the process underlying the reduced CV of lengths with age observed in the data is growth compensation, size-selective mortality, or a consequence of the decreasing slope of the von Bertalanffy curve is not known.

The fraction of flounder mature by age (H_i) is determined from mean length at age:

$$(36)\quad H_i = \begin{cases} 1.15/(1.0 + (0.02318 \cdot L_i - 8.2972)^2) - 0.07 & \text{for } L < 350 \text{ mm} \\ 1.0 & \text{for } L \geq 350 \text{ mm} \end{cases}$$

Equation 36 was determined by fitting a function relating the fraction mature by age to mean length at age (Northeast Utilities Service Company 1987, 1992). For input into the YOY model, the total number of female spawners is $\Sigma n_i H_i$ and the length distribution is constructed from the mean lengths at age and the $n_i H_i$ females in each age-class. Values for s_i by age-class are 0.7, 0.6, and 0.45 for $i \leq 3$, and 0.4 for $i \geq 4$. These values are based on a sum of natural mortality (assumed 0.35·year^{-1}) and fishing mortality (adjusted for gear vulnerability and discard catch) estimated for the Niantic River winter flounder stock for the late 1980s (Northeast Utilities Service Company 1992).

Numerical considerations

To reduce computational demands, a sample of individual larvae, rather than every larva, is followed in the model compartment. For each simulation, we select a fraction (N/TOT) of each female's surviving first-feeding larvae, where N is the number of model individuals followed and TOT is the total number of surviving first-feeding larvae. TOT is obtained by summing over all female egg cohorts. When introduced into the population, each model individual j represents m_j (=TOT/N) other identical population individuals in the compartment. Each model individual is rescaled to the number of population individuals it represents for computing the amount of prey consumed (eq. 28) and expressing densities (e.g., number per square metre).

To reduce demands on computer memory, we used a resampling algorithm for all simulations except those for identifying characteristics of survivors. The resampling algorithm permits sufficient survival to the end of the simulation for interpretation of model predictions while not having to initially follow thousands of larvae. Resampling involves following a constant number of model individuals by randomly selecting replacements for those that die on each day (see Rose et al. 1993*b*). The donor individuals are randomly selected from those alive on that day and m_j for the selected donor is adjusted to reflect the fact that mortality has occurred. All of the attributes of the dead individual are replaced with those of the donor individual. We used resampling with 1000 model individuals for model simulations presented in this paper.

To determine any differentiating characteristics of survivors, we performed two simulations using $N = 10^5$ initial first-feeding larvae without resampling. Resampling was not used because it would not permit daily tracking of the histories of those individuals that ultimately survive to age 1. Without resampling, as model individuals died, they and the population individuals they represented were simply removed from the simulation. The first simulation computed average values of individual-related characteristics for all individuals (survivors and nonsurvivors) and noted which of the first-feeding larvae survived to age 1. The second simulation repeated the first exactly, except that average values of characteristics only for those individuals that survived to age 1 were computed.

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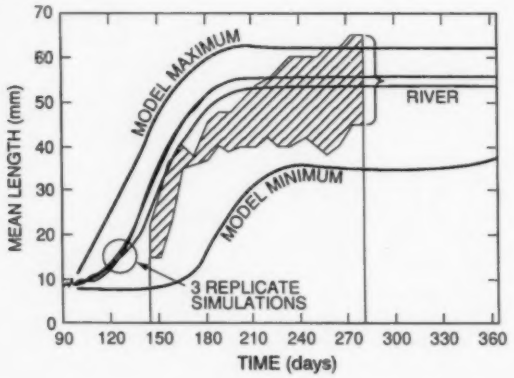
Table 3. Predicted life stage specific durations, mortality rates, survival, growth rates, and abundances (numbers entering) for the three replicate base-line simulations and their averages.

Stage	Replicate	Duration (days)	Mortality rate (day^{-1})	% Survival	Growth rate ($mm \cdot day^{-1}$)	Number entering ($\times 10^5$)
Egg	1	29.5	0.190	0.41		1240
	2	29.6	0.180	0.43		1190
	3	29.5	0.190	0.41		1210
	Mean	29.5	0.190	0.42		1627
Yolk-sac larva	1	9.0	0.160	23.2		5.12
	2	8.4	0.160	25.1		5.13
	3	9.0	0.160	23.3		4.97
	Mean	8.8	0.160	23.9		5.07
Larva	1	48.8	0.024	30.8	0.080	1.19
	2	52.1	0.024	27.9	0.075	1.29
	3	47.6	0.025	30.8	0.081	1.15
	Mean	49.5	0.024	29.8	0.079	1.21
Juvenile (growing season)	1	162.2	0.015	9.0	0.320	0.37
	2	161.0	0.016	8.0	0.320	0.36
	3	154.3	0.014	10.8	0.320	0.36
	Mean	159.2	0.015	9.1	0.320	0.36
Juvenile	1	250.1	0.012	5.3	0.200	0.37
	2	251.2	0.012	4.6	0.200	0.36
	3	244.8	0.011	6.1	0.200	0.36
	Mean	248.7	0.012	5.3	0.200	0.36
Age 1	1					0.019
	2					0.017
	3					0.022
	Mean					0.019

Note: The juvenile growing season stage is defined as metamorphosis to October 1.

Fig. 7. Mean lengths of winter flounder juveniles during their 1st year of life. The means and extremes from the three base-line simulations are shown along with the observed range in mean lengths of winter flounder from the Niantic River. The observed lengths are based on weekly collections at two river stations in 1988–1991 (see Northeast Utilities Service Company 1992 for details).



Predictions from simulations with and without resampling are indistinguishable, with more variability generated among replicate simulations (different random number seeds) than between simulations that differed on the basis of whether or not resampling was used.

YOY model results

Base-line conditions

Three replicate simulations of the YOY model with 225 female spawners were performed and the predictions compared with observed values from the Niantic River and Bay for 1983–1991. The number of female spawners was set to 225 because long-term (200 year) simulations under base-line conditions stabilized at about 225 female spawners per year (see below, Fig. 11*a*). The lengths of females were generated from a triangular distribution with minimum = 170 mm, mode = 300 mm, and maximum = 450 mm (Northeast Utilities Service Company 1992). Replicate simulations used different random number sequences that affected the sizes of female spawners, stochasticity in daily temperatures, realized prey encounters and captures, and realized probabilities of dying.

The YOY model generated densities of larvae and juveniles that were comparable in magnitude and timing with those observed in the Niantic River (Fig. 5). Larvae appeared in mid-February (day 42), peaked near the end of March (days 74–92) at about $2.5 \cdot m^{-3}$, and disappeared by the end of May (days 140–160) because of mortality or metamorphosis into juveniles. Juveniles showed peak average densities of about $0.5 \cdot m^{-3}$, which occurred about 1 month earlier than peak densities observed in the field (Fig. 6). Field data likely miss the peak density because of difficulties in sampling newly metamorphosed juveniles. It takes several weeks for the newly metamorphosed juveniles to aggregate in the nursery areas sampled. Some 20- to 30-mm juveniles were obtained in the

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Fig. 8. Proportion of winter flounder metamorphosing per day for each of the three base-line simulations. Shown are all individuals that metamorphosed (all), those that also survived to the end of the year (survivors), and the difference between these groups (bias) that highlights the periods when settlement has advantages (positive bias) or disadvantages (negative bias).

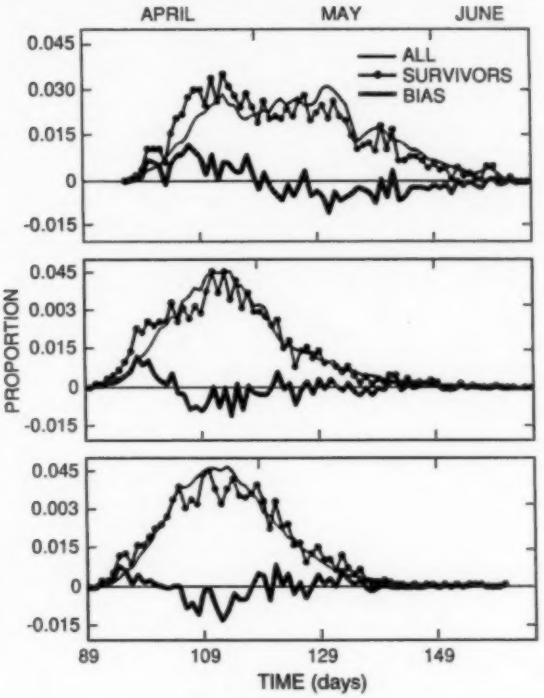

Fig. 9. Comparison of predicted recruitment (survivors to age 1) for 1977–1987 from three replicate simulations with the observed recruitment in the Niantic River. Simulations were performed for each year using observed numbers of spawners and daily temperatures. Predicted and observed numbers of spawners and recruits were standardized by dividing each by their respective mean values. The second set of 1977 predictions (symbols inside squares) resulted from a doubling of zooplankton densities.

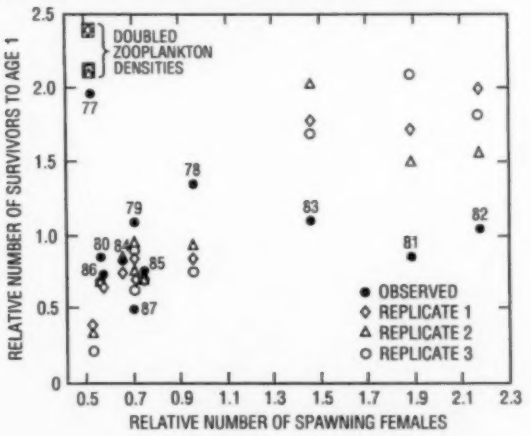

Table 4. Spawner–recruit data for 1977–1987 in the Niantic River (Northeast Utilities Service Company 1993).

Year-class	Female spawners	Ratio of female spawners to mean	Female recruits	Ratio of female recruits to mean
1977	20 097	0.53	65 044	1.93
1978	36 544	0.97	45 370	1.35
1979	27 262	0.72	36 547	1.09
1980	21 608	0.57	28 584	0.85
1981	70 441	1.86	28 347	0.84
1982	81 324	2.15	34 745	1.03
1983	55 104	1.46	36 825	1.09
1984	25 544	0.68	28 012	0.83
1985	28 789	0.76	25 235	0.75
1986	22 241	0.59	24 915	0.74
1987	27 076	0.72	16 630	0.49
Mean	37 821		33 659	

Note: Estimates of the number of female spawners are based on annual measurements of adults during the spawning season. Estimates of the number of recruits represent the expected number of female spawners for that year-class over its lifetime.

early samples (late May), implying that these individuals settled some time prior to the initiation of sampling.

Model predictions of life stage specific duration, growth rate, mortality rate, and survival (Table 3) were also comparable with field estimates. Mortality rates of yolk-sac larvae and larvae were set in the model to obtain survival rates from hatching to metamorphosis of ≈7.0% to agree with observed survival rates of 2.1–17.6% for 1984–1991 in the Niantic River. Survival rates of YOY juveniles in the Niantic River from May 15 (day 135) to September 30 (day 273) for 1983–1989 and in 1991 ranged from 6.0 to 20.0%; an unusually high survival rate of near 100% was observed in 1988 and an unusually low survival rate of 0.4% was observed in 1990 (Northeast Utilities Service Company 1992). Survival of juveniles over the growing season ranged from 8.0 to 10.8% in the simulation. Field estimates of larval growth rates for 1983–1991, based on the progression of mean weekly lengths, averaged 0.10 mm·day^{-1} (range 0.084–0.123) in the Niantic River and averaged 0.072 mm·day^{-1} (range 0.056–0.094) in Niantic Bay. Model-predicted daily growth rates of larvae averaged 0.079 mm·day^{-1}. Mean lengths of juveniles over time were similar to field data. Most growth occurred during spring and summer and little or no growth occurred after midsummer (day 200) (Fig. 7).

The individuals surviving to the end of the year (i.e., to age 1) showed small but consistent differences in early larval growth rates and densities at metamorphosis. Compared with all individuals, those surviving to age 1 grew at slightly faster rates over their first 15 days of feeding (replicate averages of 0.043, 0.045, and 0.051 mm·day^{-1} versus 0.041, 0.041, and 0.048 mm·day^{-1}) and metamorphosed when densities were generally lower (0.24, 0.38, and 0.30·m^{-2} versus 0.27, 0.39, and 0.32·m^{-2}). We confirmed these differences by performing seven additional replicate base-line simulations. The results were consistent with those from the first three simulations; in each case, survivors to age 1 were consistently those with faster growth rates at early ages and those that settled at lower densities. The differences in growth rates result in differences in length of <0.1 mm at 15 days from first feeding. Metamor-

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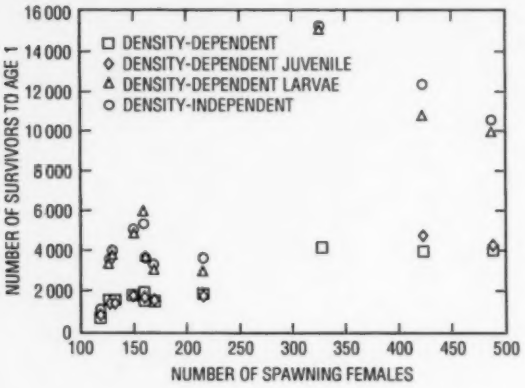
Fig. 10. Predicted numbers of recruits (survivors to age 1) for simulations under base-line conditions and with three combinations of density-dependent feedbacks removed. □, density dependence operating in both larval and juvenile stages (base-line conditions); ◇, density dependence operating during the juvenile stage only (i.e., no larval affect on zooplankton); △, density dependence operating during the larval stage (i.e., no juvenile effect on benthos and no density-dependent mortality of juveniles); ○, density independence.



phosing when densities are 0.24 versus 0.27·m^{-2} results in mortality rates of 0.163 and 0.185·day^{-1}, respectively, or probabilities of dying of 0.85 versus 0.83, respectively. The small differences in early larval growth rate and densities at metamorphosis between all individuals and those that survive to age 1 demonstrate the subtlety of survival selection in model simulations.

Although most survivors to age 1 metamorphosed during the middle of the metamorphosis period when densities were highest, the differences in fractions metamorphosing between survivors and all individuals (bias) were consistently more than expected (positive bias) during the early period of metamorphosis and consistently less than expected (negative bias) during the middle period of metamorphosis (Fig. 8). Metamorphosing when densities are low is by itself not sufficient to ensure higher survival. Individuals that metamorphosed late, when densities were also low, tended to survive in proportion to their numbers (zero bias).

YOY model corroboration

We compared the number of age-1 survivors in the YOY model with the observed levels of recruitment for 11 years of data from the Niantic River population. The available data consisted of daily temperatures, the estimated annual number of female spawners, and the estimated number of recruits from 1977 to 1987 (Table 4). Recruits were estimated from adult surveys and are expressed as the expected number of spawning females resulting from that year-class over their lifetime. Numbers of female spawners and temperatures in simulations were varied to reflect observed values for each of the 11 years. Specifically, the numbers of female spawners were increased or decreased from the base-line value of 225 in proportion to the number of observed females in each year relative to their overall average. This resulted in the numbers of spawning females ranging from ≈0.5 to 2 times the base-line level (Table 4). Observed daily temperatures for each year were directly used in model simulations (linear interpolation was used to estimate missing values). All other model inputs (e.g., zooplankton, size distribution of spawners) were maintained at their base-line values. To permit easy comparison, we expressed predicted and observed recruitment as relative indices by dividing each by their respective mean values. Three replicate simulations of each year were performed using different random number sequences.

Both observed levels and model predictions suggest a ceiling on recruitment in years of high numbers of spawners (Fig. 9), although model predictions were consistently higher. For the remaining years of low to average observed recruitment, the predicted recruitment was also low to average. Predicted survival rates of larvae and juveniles for the 3 years of high levels of spawning were lower than those for the remaining years (larvae: 21.4 vs. 25.8%; juveniles: 4.3 vs. 5.9%, respectively). Mortality rates of other stages did not differ consistently between the 3 years of high spawning and the remaining years.

For 1 year, 1977, the observed and predicted recruitment differed greatly (Fig. 9). Several aspects of 1977 were exceptional. Among the 11 years, 1977 had the lowest number of spawners, the coldest February, and the highest estimated recruitment. The YOY model can predict recruitment similar to that observed in 1977 by doubling the zooplankton densities but not by decreasing stage-specific mortality rates. Under the conditions of doubled zooplankton densities, the level of recruitment predicted for 1977 (2.0–2.4; Fig. 9) was reasonable, and the larval growth and survival rates from hatch to metamorphosis (0.11 mm·day^{-1} and 10.3–10.9%, respectively) remained within acceptable values. Other hypotheses have been proposed to explain why years with cold spring temperatures benefit winter flounder. These include juveniles benefitting from increased organic matter going to the benthos as the result of a prolonged algal bloom (zooplankton are more delayed by cold temperatures than phytoplankton) and cold temperatures reducing mortality of newly settled winter flounder (Jeffries and Tercerio 1985) by increasing the mortality rate of predatory *Crangon* shrimp or forcing them to leave winter flounder nursery areas (Northeast Utilities Service Company 1994). However, independently halving the mortality rates of eggs, yolk-sac larvae, larvae, or juveniles resulted in relative recruitment indices of <0.5, which is lower than the observed value of 1.9. Annual average copepod densities (based on use of 333-μm mesh) at two Niantic Bay stations in 1977 were the second and third highest during 1976–1983 (Northeast Utilities Service Company 1984). Annual average copepod densities based on use of 333-μm mesh may not be a good indicator of available food because winter flounder eat zooplankton smaller than those captured by a 333-μm mesh and then only during spring. Although zooplankton production dynamics are tightly coupled with temperature (Durbin and Durbin 1981; Verity 1986; Huntley and Lopez 1992), we cannot determine whether the pattern of temperature increase during the spring of 1977 would have resulted in a doubling of the densities of suitably small zooplankton.

YOY density dependence

To examine the degree of density dependence in model

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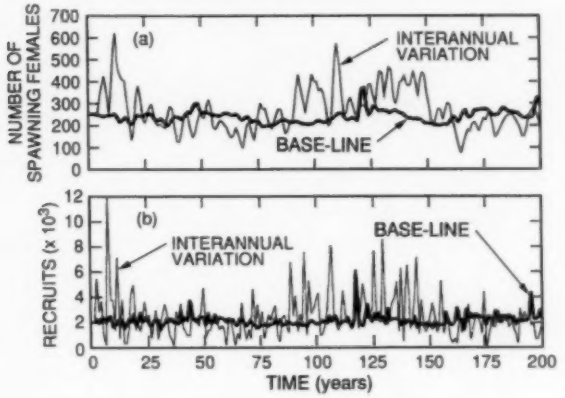
Fig. 11. Annual number of (*a*) female spawners and (*b*) total recruits for 200-year simulations for base-line conditions and for interannual variation imposed on the factors of daily temperatures, prey levels, and larval and juvenile mortality rates.



simulations, we repeated the 11 1-year simulations with temperature regimes and numbers of spawners based on the Niantic River data but with various combinations of the density-dependence feedbacks in the model removed. Simulations were performed with either no density dependence operating during the larval stage (i.e., no effect of flounder consumption on zooplankton densities), none operating during the juvenile stage (i.e., no effect on benthos and no density-dependent mortality), or no density dependence in either the larval and juvenile stages. The feedback on prey was removed for larvae or juveniles by setting $EAT_{i,t} = 0$ in eq. 27. The density-dependent component of juvenile mortality was eliminated by setting ZFAC = 1.0 for juveniles <20 mm in length in eq. 30. We report the average of three replicate simulations of each of the 11 years with larvae, juveniles, or both stages being modelled without density dependence. Recruitment is expressed as the predicted number of survivors to age 1.

The effect of density dependence on the number of survivors to age 1 was greatest during the juvenile stage, especially for high numbers of spawners (Fig. 10). Recruitment under complete density independence and density dependence in the larval stage only were similar to each other. Further, both conditions predicted recruitment that was consistently higher (about 2.0- to 3.5-fold) for years of high numbers of spawners than when density dependence was operating in both stages or when juveniles only experienced density dependence. The relatively high degree of density dependence predicted may be an underestimate. Model predictions of recruitment for the 3 years of high numbers of spawners were consistently higher than the observed levels of recruitment (Fig. 9), implying that a greater degree of density dependence in the simulations is needed for predicted recruitment to better match the observed recruitment.

Long-term simulation results

To identify factors operating during the 1st year of life that are important to recruitment, we performed two 200-year simulations of the coupled YOY and adult matrix models. The first simulation used all base-line values and showed that model predictions were stable over time. The second simulation evaluated interannual variation in temperature, zooplankton, benthos, larval mortality rate, and juvenile mortality rate. These factors were varied to match as closely as possible the observed variation in the Niantic River.

Temperatures were varied by generating a random number from –2 to 2 for each year, and adding this value to daily temperatures. Over 90% of the daily temperatures from 1977 to 1988 measured in Niantic Bay were within 2°C of their long-term mean daily temperature.

Prey levels were varied by multiplying daily equilibrium densities each year by a random number from 0.6 to 1.4 for zooplankton (2.3-fold) and 0.9 to 1.1 for benthos. Mean weekly densities of zooplankton in Niantic Bay across years (1976–1979) during the larval period of winter flounder (March through June) varied from 1.5-fold (maximum/minimum) to 10-fold, with most weeks showing about a 3-fold variation across years (Northeast Utilities Service Company 1980). Annual average copepod densities varied 2.5- and 3.4-fold at two Niantic Bay stations for 1976–1983 (Northeast Utilities Service Company 1984). Empirical data on interannual variation in benthos are difficult to evaluate because of the diversity of model prey types included in our benthos prey groups and because observed data tend to be gear and site specific (Rudnick et al. 1985; Frithsen 1989). In lieu of good empirical information, we performed model simulations to identify a range of benthos densities that resulted in a realistic range of winter flounder sizes. Varying benthos by using a multiplier of 0.9–1.1 resulted in mean lengths of winter flounder at the end of the growing season ranging from 38 to 64 mm over the 200 years of simulation. This is similar to the range of 42–66 mm observed from 1983 to 1991 in the Niantic River (Northeast Utilities Service Company 1992).

Mortality of larvae and the density-dependent portion of juvenile mortality (8–20 mm in length) was varied year to year by multiplying the daily probability of dying of each individual by a factor that ranged from 0.9 to 1.1. The jellyfish *Cyanea* sp., a potentially important predator of winter flounder larvae, varied fivefold over 14 years in the Niantic River (Brewer 1989); information on interannual variation in abundances of juvenile predators such as *Crangon* shrimp are unavailable. Varying the probability of dying of larvae and juveniles by 0.9–1.1 resulted in survival rates that ranged from 0.05 to 10.9% for the larval stage (hatching to metamorphosis) and from 2.9 to 29.6% for juveniles from metamorphosis to the end of the growing season. Some of these larval survival rates extend beyond the range observed during 1983–1991 in the Niantic River (2.1–13.5%) (Northeast Utilities Service Company 1992), but 75% of the predicted values are within the observed range. Based on catch-curve analysis of data at two stations in the Niantic River, the survival rates for juveniles (May to September) varied from 6.0 to 20.0%, with unusual values of ≈100% in 1988 and 0.4% in 1990 (Northeast Utilities Service Company 1992).

The predicted number of female spawners and the total number of recruits to age 1 were stable over time for both the base-line and interannual variation simulations (Fig. 11). CVs of the number of spawners (35%) and of recruits (71%) for the interannual variation simulation were similar to or slightly less

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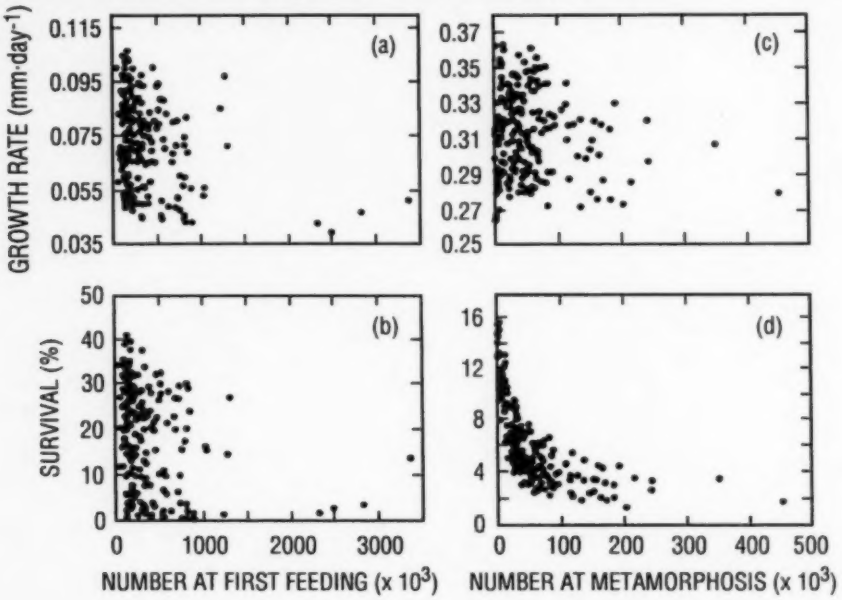
Fig. 12. Average growth rate and survival through a life stage versus number entering the life stage for the 200-year interannual variation simulation: (*a*) larval growth rate, (*b*) larval survival, (*c*) juvenile growth rate, and (*d*) juvenile survival.



than the CVs observed for winter flounder in Niantic River and Narragansett Bay, and for other flatfish. CVs based on data from 1976 to 1991 in the Niantic River were 53% for female spawners and 68–84% for three different indices of recruitment (Northeast Utilities Service Company 1992). Jeffries et al. (1988) reported CVs of 45–75% for four different time series (14–28 years duration) of winter flounder in the Narragansett Bay area. Three of their indices were based on summed quarterly or monthly means and one index was based on mean fall trawl densities. In other flatfish species, CVs of recruitment varied from 38 to 68% for four of five species; the fifth, European sole (*Solea solea*) in the North Sea, had a CV of 117% (Rothschild and DiNardo 1987). Considering that only a subset of possible factors affecting recruitment were varied from year to year in our interannual simulation, prediction of somewhat lower CVs than observed for natural populations is expected.

Our model analyses suggest that recruitment is coarsely set at the larval stage. Zooplankton levels, and to a lesser extent the number of eggs spawned and the temperature regime, affect recruitment. The predicted number of age-1 recruits was correlated with the number of larvae that reached metamorphosis ($R^2 = 0.71$) but not with the number of eggs, hatchlings, or first-feeding larvae ($R^2 < 0.18$). Further, the number that metamorphosed was only marginally related to the number of eggs, hatchlings, or first-feeding larvae ($R^2 < 0.31$). We related the number of recruits and the number metamorphosing to the number of eggs spawned (EGGS) and the temperature (TEMP), zooplankton (ZOOP), benthos, larval mortality, and juvenile mortality (JMORT) factors using stepwise linear regression (SAS Institute Inc. 1988). The final regression models, which include all terms with partial R^2 (shown in parentheses) >0.05 are

$$\text{(37)}\quad \text{number metamorphosing} = -146\,871.8 + 153\,820.6 \cdot \text{ZOOP} + 19173.6 \cdot \text{TEMP} + 0.0003 \cdot \text{EGGS} \quad (0.31)\ (0.15)\ (0.09)$$

$$\text{(38)}\quad \text{number of recruits} = 4999.8 + 4230.6 \cdot \text{ZOOP} + 0.000\,0086 \cdot \text{EGGS} - 8251.2 \cdot \text{JMORT} + 398.27 \cdot \text{TEMP} \quad (0.28)\ (0.11)\ (0.09)\ (0.07)$$

The model R^2 (the sum of partial R^2 values) is 0.56 for the number metamorphosing and 0.55 for the number of recruits. The most important factor was zooplankton densities for both the number metamorphosing and, through effects on larvae, the number of recruits. The importance of zooplankton densities in the regressions implies that winter flounder larvae are food limited (larvae do not obtain their maximum possible consumption rate every day), not necessarily that larvae affect the densities of zooplankton through their consumption rates (see below). Number of eggs and the remaining factors of temperature and juvenile mortality rate, while significant contributors, were of secondary importance. The factors of larval mortality rate and benthos densities were unimportant in the regressions. Applying the regression models (eqs. 37 and 38) without the zooplankton factor resulted in model R^2 values less than one half of those for models that included interannual variations in zooplankton density (total $R^2 = 0.23$ versus 0.56, and 0.22 versus 0.55, for number metamorphosing and recruiting, respectively).

Survival during the juvenile stage was strongly density dependent (as expected from the model formulation), while density-dependent survival of larvae and growth of both juveniles and larvae appeared as threshold responses (Fig. 12). Survival and

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growth rates of larvae were variable for low to average numbers of first-feeding larvae and low for high numbers of first feeders (Figs. 12*a* and 12*b*). Average growth and survival rates of larvae in years with $<10^6$ first-feeding larvae were 0.072 mm·day^{-1} and 19.0%, which are greater than the 0.061 mm·day^{-1} and 10.5% obtained for the 8 years with $>10^6$ first-feeding larvae. Larval mortality of winter flounder in the Niantic River varied directly with egg production ($R^2 = 0.48$) whereas larval growth rate varied inversely with the number of first-feeding larvae ($R^2 = 0.59$) (Northeast Utilities Service Company 1992). Juvenile growth rate over the growing season was also variable for years of low to average numbers metamorphosing and low when high numbers metamorphosed (Fig. 12*c*). Mortality of juveniles 8.0–20.0 mm was formulated explicitly as being density dependent (eqs. 30–32); thus, as expected, juvenile survival rate varied inversely with the numbers metamorphosing (Fig. 12*d*).

Discussion

Coupled YOY and adult models approach

One major difference between previous approaches and the one presented herein is the greater detail in our YOY model. Coupled YOY and adult age-structured matrix models have been used extensively to simulate fish population dynamics (e.g., DeAngelis et al. 1977; 1980; O'Neill et al. 1981; Goodyear 1985; Schaaf et al. 1987; Saila et al. 1991; Northeast Utilities Service Company 1992). In all of these applications, the critical 1st-year survival rate was treated in an aggregate manner either by being solved for to ensure population stability or by being represented with a spawner–recruit relationship. Solving for survival rate preempts analysis of the processes affecting recruitment that operate during the critical 1st year of life. Specifying a spawner–recruit relationship is usually difficult because required data should span multiple generations and, to be representative, should include a wide range of biological and environmental conditions. O'Neill et al. (1981) provide some resolution within the YOY component of their model by dividing the YOY into six life stages (egg, yolk-sac larva, larva, and three juvenile stages). Mortality was density independent for the egg and two of the juvenile stages; survival through the other stages was represented by functional forms similar to those used in spawner–recruit relationships. In our approach, we follow spawners and their progeny as individuals on a daily time step through their 1st year of life.

We view the detailed YOY simulation and the spawner–recruit approaches to 1st-year survival as complementary. Each offers advantages depending on the nature of available data and the purpose of the analysis. The advantage of the detailed YOY approach is that 1st-year survival is dynamically simulated without a priori specification of a spawner–recruit curve. Consequently, there is much greater flexibility in the questions that can be addressed. The specific shape of the spawner–recruit curve results from our depictions of the main and interactive effects of temperature, prey, flounder size, bioenergetics, and ontogenetic rates. Assuming that it is properly configured, the detailed YOY model can potentially reflect the effects on recruitment of processes or events operating on individuals on the scale of days. Further, because details of temperature, spawning, life stage specific vital rates, and the levels and timing of prey abundances are explicitly represented, their direct and interactive effects on population dynamics can be assessed through exploratory simulations. Determining how a spawner–recruit curve fitted to data would change in response to previously unobserved conditions is difficult.

The disadvantage of using a detailed YOY model compared with the spawner–recruit approach is its much greater demands for data, which makes configuring a truly site-specific version difficult. The detailed YOY model requires a wide variety of information (daily temperatures; seasonal prey densities; and abundances, time courses, and vital rates of the fish of interest within the year), preferably for multiple years. Although the annual nature of spawner–recruit data implies that many years of observation are required, data on the numbers of spawners and recruits are more commonly measured in many systems. The relatively narrow scope of data (annual values of spawners and recruits) can make the spawner–recruit approach easier to justify as being more site specific and empirically based. As one progresses from the spawner–recruit to the detailed YOY approach, there is a tradeoff between more detail and resolution in the YOY life stages and increasing uncertainty in each component of the YOY model as data are extended beyond their original context. Although we believe that the model presented here for winter flounder is well suited for exploratory analyses, we would argue that additional data are required for site-specific impact and risk assessment. A spawner–recruit approach has been used successfully as part of an analysis of the impacts of the Millstone Nuclear Power Station on the winter flounder population (Northeast Utilities Service Company 1992, 1993, 1994). The extensive data base developed for the winter flounder population in the Niantic River offers an opportunity for applying both the detailed and spawner–recruit approaches.

A second difference between the usual age-structured adult model and our approach is the fecundity relationship. The classical approach represents fecundity as the product of the fraction of fish that are mature times number of eggs per individual, both assumed to be strict functions of age. In our approach, fecundity is time dependent and can vary as a function of age, temperature at spawning, and size-dependent maturity. Use of the inverse of the von Bertalanffy growth curve (eqs. 34 and 35) maintains differences in mean lengths at age 1 that, while diminishing with age, persist from year to year throughout an individual's adult life. For example, predicted variation in mean lengths at age 1 of 38–62 mm from the 200-year interannual variation simulation translates into differences in lifetime fecundity of 540 802 eggs for a female that was 38 mm at the end of the 1st year of life to 602 045 eggs for a female that was 62 mm, or an increased lifetime fecundity of 23%. Size at first feeding also varies as a function of female size and temperature at spawning (eqs. 4 and 7). A female of average size (300 mm) spawning early in the season (at 2°C) would produce 540 802 eggs and 4.33-mm first feeders compared with the same female, spawning late in the season (at 5°C), producing 576 948 eggs and 4.17-mm first feeders. Effects of female age, female size, or temperature on egg viability and hatching success, while not represented in this version, could also be easily incorporated into our adult model. Allowing fecundity, size at first feeding, egg viability, or hatching success to depend on female size and water temperature could have important consequences on model dynamics that are not easily analyzed with models that use fixed length at age and maturation at age schedules.

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Winter flounder population dynamics

Model predictions of within-year YOY densities and growth and mortality rates are within the range observed for winter flounder in the Niantic River and Bay. Both model predictions and 11 years of spawner–recruit data showed a ceiling on recruitment under high numbers of spawners. Multiple-year simulations using the coupled YOY and adult models generated somewhat less variable levels of spawners and recruits (as expected) compared with available long-term records.

A fundamental assumption underlying the analyses presented in this paper is that the population dynamics of winter flounder can be adequately simulated using a single, well-mixed compartment. Spatial heterogeneity in zooplankton is implicitly represented by using CDFs (rather than Poisson distributions) to relate realized encounters to the mean encounter rate. However, CDFs simulate fine-scale patchiness only; individual larvae in the model do not have any memory from day to day. Thus, a larva realizing above-average zooplankton densities one day (i.e., multiplier of mean >1) has no effect on its likelihood of experiencing above-average densities on the next day. This could occur if zooplankton patches were small such that larvae entered and exited a patch on a daily time scale. Broader scale patches that would permit a larva to remain in a patch for several successive days are not represented by the use of a CDF. The spatial scales of prey patches relative to individual larval movement distances are unknown. There also may be important differences in the environment within and between the river and bay that are not explicitly represented in a one-compartment model. Both larvae and juveniles are found in the Niantic River and Bay. At present, the model assumes complete spawning fidelity by females; all spawners were themselves spawned, and continue to spawn, in the one compartment. Although the potential for mixing of adult stocks offshore exists, adults appear to exhibit a high degree of fidelity to their natal estuary (Klein-MacPhee 1978). However, indirect evidence based on mass-balance calculations showed that 65–89% of winter flounder larvae entrained at the Millstone Nuclear Power Station in Niantic Bay could have originated from spawning in other areas of Long Island Sound (Northeast Utilities Service Company 1994), as opposed to our one-compartment assumption that they originated in the Niantic River. With adequate information, multiple compartments for YOY life stages and a more complicated adult model involving stock mixing are feasible. In lieu of such data, we view a single, well-mixed compartment as a reasonable working assumption.

Model simulations indicate that recruitment is largely set by metamorphosis, with density-dependent growth and survival operating during the larval and juvenile stages. Our evaluation of the role of density dependence showed that predicted recruitment (number of age-1 survivors) for years of high numbers of spawners without density dependence would be two to three times higher than with density dependence (Fig. 10). Analysis of the 200-year interannual variation simulation revealed recruitment to be correlated with the number metamorphosing but not with abundances at prior life stages. Also, larval growth and survival and juvenile growth were variable at low to average densities and low at high densities (Figs. 12*a*–12*c*). The model was configured such that consumption of prey can be offset by prey turnover up to point, but at sufficiently high consumption rates, prey are reduced below equilibrium densities. Slower growth and higher mortality (mortality decreases with flounder size; Fig. 4*a*) are consequences of reduced prey densities. Juvenile survival in the interannual variation simulation was a monotonically decreasing function of density (Fig. 12*d*) because the density-dependent component of mortality was specified as a continuous function of density (ZFAC; eq. 32 and Fig. 4*b*). Others have also concluded or implied that density dependence in winter flounder populations likely occurs in several YOY life stages (Pearcy 1962*b*; Northeast Utilities Service Company 1992). van der Veer (1986) concluded that recruitment of European plaice in the Wadden Sea is coarsely set in the offshore larval stage by environmental factors and fine tuned via density-dependent mortality during the early juvenile stage. Analyses of marine fishes (Houde 1987, 1994) and marine demersal fishes (Myers and Cadigan 1993) also imply that year-class strength is likely to be coarsely established in the larval stage, with density-dependent mortality likely being more important in the juvenile stage.

Individuals growing faster during the early larval stage and metamorphosing early in the season when juvenile densities are relatively low have a survival advantage in model simulations. Faster growth of winter flounder during the early larval stage permits individuals to progress more rapidly through early life stages, when the mortality rate is generally higher, and metamorphose sooner (Chambers and Leggett 1987). In an analysis of vital rates of five fish species, Houde (1987) found that slight variations in larval growth rates could greatly affect survival. Al-Hossaini et al. (1989) reported that early cohorts of juvenile European plaice had faster growth rates, as inferred from otolith increment analysis, but also lower survival rates than later cohorts. The small magnitude of differences in simulations between survivors and all individuals in their early larval growth rates (<0.005 mm·day^{-1}) and densities at metamorphosis ($<0.03 \cdot m^{-2}$) implies that detection of such differences in field data would be difficult. Determination of dates of metamorphosis and within-year cohort mortality rates may be a more practical means of identifying survival advantages to individuals.

On the basis of our analyses, future monitoring of the status of winter flounder populations should focus on temperature, zooplankton, spawning stock size, larval and recruitment indices, and detailed study of early juveniles and their interaction with predators. While information on egg production is needed to understand spawner–recruit dynamics, egg production alone is inadequate. An index of the number metamorphosing (such as the 7-mm index used for the Niantic River; Northeast Utilities Service Company 1992) would provide a coarse indicator of recruitment. Others have proposed that the late-larval to early juvenile life stages are the earliest stages that are strongly related to recruitment in marine species (Sissenwine 1984; Peterman et al. 1988; Bradford 1992; Myers and Cadigan 1993). Comparing the numbers metamorphosing with the numbers at age 1 would permit inference on the relative role of larval and juvenile stages to recruitment. The density and timing of small zooplankton during the time when larval winter flounder are present would provide information on an explanatory variable that could be used to adjust the larval index for interannual differences in prey. The numbers surviving to metamorphosis and to recruitment were most closely related to zooplankton levels in model simulations ($R^2 \approx 0.3$), and secondarily to the number of eggs produced and temperature

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(eqs. 37 and 38). The most feasible model explanation for the extraordinarily high recruitment in 1977 was a doubling of zooplankton densities. However, we cannot rule out the effects of low winter temperatures reducing predation pressure on early (8.0–20.0 mm) juveniles (Jeffries and Terceiro 1985; Northeast Utilities Service Company 1994). Once the effects of dominant factors (e.g., zooplankton) are first removed, better estimates of the effects of anthropogenic factors affecting larvae, such as power plant entrainment (Northeast Utilities Service Company 1992) and the presence of toxic substances (Nelson et al. 1991), may be possible. Detailed study of early juveniles and their interaction spatially and temporally with invertebrate predators such as *Crangon* shrimp would aid in better specifying the relationship between survival rate and density for recently settled juvenile winter flounder. We presently rely heavily on data from European plaice in the Wadden Sea (van der Veer 1986) to specify the variation in mortality rate as a function of juvenile density (eq. 32; Fig. 4*b*).

Further elaborations of the model could involve representing spatial heterogeneity explicitly, fishing mortality in more detail, and density-dependent aspects of reproduction. Configuration of multiple model boxes would require information on differences in the environments of each of the boxes and rules for moving fish among the boxes. More detailed representation of interannual differences in fishing mortality could add realism to model simulations. Although fishing mortality is held constant for all multiple-year simulations, the actual instantaneous rate of fishing mortality has varied from about 0.4·year^{-1} in the 1960s to 1.3·year^{-1} in 1991 (Northeast Utilities Service Company 1994). Fishing has a large effect on adult winter flounder dynamics because it is the single largest source of adult flounder mortality. Because of low winter flounder stocks, further fishery management actions are likely (Howell et al. 1992). Fishing mortality is also a potentially important density-dependent process, perhaps exhibiting logistic-like behavior as a function of fishable stock (Van Winkle et al. 1978). Further elaboration of reproduction, beyond the effects of differences in mean length of YOY and size-dependent maturation, could focus on the possible density-dependent aspects of the allocation of energy between somatic growth and reproductive products. Fecundity has been related to energy intake for winter flounder (Tyler and Dunn 1976) and plaice (Rijnsdorp 1990), and a general model of energy allocation between somatic growth and reproductive products has been proposed using data on American plaice (*Hippoglossoides platessoides*) (Roff 1983). Whether these model elaborations would increase the predictive power of the model or simply increase model prediction uncertainty depends on their importance to the questions being addressed and our confidence in their formulations.

Acknowledgements

We thank Larry Buckley of the National Marine Fisheries Service and Ernesto Lorda, Dale Miller, Linda Bireley, and the rest of the staff at the Northeast Utilities Environmental Laboratory for sharing their data and thoughts with us. Larry Barnthouse, Webb Van Winkle, John Boreman, and an anonymous reviewer provided useful comments on an earlier version of this paper. The research was sponsored by the Electric Power Research Institute under contract No. RP2932-2 (DOE No. ERD-87-672) with the U.S. Department of Energy, under contract No. DE-AC05-84OR21400 with Lockheed Marietta Energy Systems, Inc.

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Phytoplankton photosynthesis at two index stations in Lake Ontario 1987–1992: assessment of the long-term response to phosphorus control

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Abstract: Phytoplankton photosynthesis was measured from May to late October at two stations in Lake Ontario from 1987 to 1992, and published data from the International Field Year for the Great Lakes (IFYGL) in 1972 were reanalyzed. Our measurements indicate that seasonal phytoplankton photosynthesis (May 1 – October 31) may have declined about 30% since IFYGL. An empirical relationship between seasonal phytoplankton photosynthesis and mean phosphorus concentration indicates that the estimate of offshore seasonal PP by the Lake Ontario Nutrient Assessment Study (LONAS) in 1982 was unusually low (44%) compared with that predicted from phosphorus concentration. Accurate assessment of long-term change in productivity has been hampered by lack of adequate base-line data from the past. Annual variability in seasonal phytoplankton photosynthesis was remarkably low at both stations during our study with the exception of anomalously low results in 1988. Long-term changes in phytoplankton photosynthesis are probably associated more with declining summer chlorophyll levels than with changes in photosynthetic capacity or its response to temperature. The importance of subepilimnetic photosynthesis, interstation differences in transparency, and seasonal changes in photosynthetic capacity as controlling factors for areal phytoplankton photosynthesis is presented. The implication of lower lake productivity for fisheries and lake management is also discussed.

Résumé : L'activité photosynthétique du phytoplancton a été mesurée de mai à la fin d'octobre en deux points du lac Ontario entre 1987 et 1992, et les données publiées obtenues dans le cadre de l'Année internationale d'étude des Grands Lacs (IFGYL), en 1972, ont fait l'objet d'une nouvelle analyse. Nos mesures semblent indiquer une réduction de 30% de l'activité photosynthétique du phytoplancton saisonnière (1 mai au 31 octobre) depuis l'IFGYL. Une relation empirique entre l'activité photosynthétique du phytoplancton saisonnière et la concentration moyenne de phosphore indique que l'estimation de l'activité photosynthétique du phytoplancton saisonnière en eau libre établie en 1982 dans le cadre de la « Lake Ontario Nutrient Assessment Study » (LONAS) était inhabituellement faible (44%) comparativement à celle déduite d'après la concentration de phosphore. Le manque de données de base historiques adéquates complique l'évaluation précise des fluctuations à long terme de la productivité. Si l'on fait exception des résultats anormalement faibles enregistrés en 1988, les fluctuations annuelles de l'activité photosynthétique du phytoplancton saisonnière observées aux deux stations tout au long de l'étude étaient remarquablement faibles. Les fluctuations à long terme de l'activité photosynthétique du phytoplancton s'expliquent probablement davantage par la diminution de chlorophylle en été que par des changements de la capacité photosynthétique ou d'une réponse à la température. L'importance de la photosynthèse subépilimnétique, les différences de transparence entre les stations et les fluctuations saisonnières de la capacité photosynthétique comme facteurs limitants pour l'activité photosynthétique du phytoplancton sont examinées. La répercussion d'une baisse de la productivité des lacs sur les pêches et l'aménagement des lacs est aussi étudiée.
[Traduit par la Rédaction]

Introduction

Over the last two decades, Lake Ontario has undergone drastic changes in two factors that regulate lake productivity and food-chain dynamics, namely phosphorus (P) loading and stocking of salmonids. Rising P concentrations in lakes Erie and Ontario up to the late 1960s and the associated problems of nutrient enrichment prompted the governments of Canada and the United States to sign the Great Lakes Water Quality Agreement in 1972. The focus of the agreement was the reduction of P loading into the Great Lakes. Offshore phytoplankton biomass in Lake Ontario was never high enough to be considered a problem in itself (Munawar and Nauwerck 1971; Glooschenko et al. 1974; Stadelmann et al. 1974; Vollenweider et al. 1974) but rising P levels were symptomatic of serious inshore problems (International Joint Commission 1969). There was widespread concern that offshore conditions could also deteriorate if P control was not implemented. As a result of the agreement, both P loadings and lakewide P concentrations have declined over the last two decades (Stevens

Received January 25, 1995. Accepted December 7, 1995.
J12735

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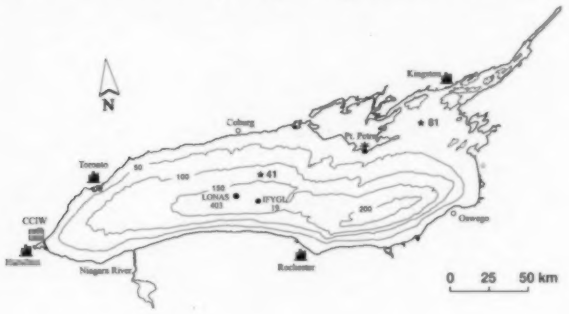
Fig. 1. Lake Ontario long-term biomonitoring station 81 (★) in the Kingston basin (depth = 34 m) and station 41 (★) in midlake (depth = 126 m), IFYGL station 19 (●), and LONAS station 403 (●). Solar radiation monitoring sites at Point Petre and the Canada Centre for Inland Waters (CCIW), Burlington, Ont., are shown.



and Neilson 1987; Neilson et al. 1994; Johengen et al. 1994). In theory this should have lowered primary productivity and the production of fish dependent on the planktonic food chain (Downing et al. 1990). Substantial reductions in inshore *Cladophora* biomass and growth rate clearly occurred as a direct result of P control (Painter and Kamaitis 1987; Painter and Jackson 1989). However, offshore phytoplankton biomass apparently had not responded to the decrease in P concentrations by 1982 (Gray 1987; Stevens and Neilson 1987). Recent information (1981 to present) has shown a long-term downward trend in both P and chlorophyll *a* (Chl) concentrations since the early 1980s (Johengen et al. 1994). Coincident with declining P loading, salmonid stocking programs have added top predators to the lake in unprecedented numbers, increasing from just over 1 million salmonids stocked in 1972 to 8.2 million in 1984 (Neilson et al. 1994).

This paper reports on a multiyear (1987–1992) study of phytoplankton photosynthesis (PP), a component of the long-term Lake Ontario Biomonitoring Project (Bioindex Project, Fisheries and Oceans Canada, Burlington, Ont.) that was initiated in 1981 to assess seasonal and long-term trends in biomass, species composition, and production at lower trophic levels in Lake Ontario (Johannsson et al. 1985; Johengen et al. 1994). Seasonal and annual trends in physical, chemical, and biological variables have been studied since 1981 by frequently sampling a few permanent stations. Surveillance projects have traditionally been infrequent and focused solely on water quality. Consequently, scientists have not been in a position to adequately address the concerns of fisheries managers about changing lake productivity. The main goals of the phytoplankton productivity part of the Bioindex Project were to determine current levels and annual variability in seasonal photosynthesis to assess whether productivity had declined in the 20 years since the International Field Year for the Great Lakes (IFYGL) in response to P control. Establishing seasonal trends and mean levels of determinants of the photosynthetic system such as Chl, light extinction, and photosynthetic parameters was considered essential for explaining current and past levels of seasonal PP. The relative importance of light versus P in controlling seasonal phytoplankton growth is reported in a companion paper (Millard et al. 1996).

Methods

Field sampling

Two locations have been sampled extensively on a weekly basis since 1981 as part of the Lake Ontario Bioindex Project (Johannsson et al. 1985) (Fig. 1). Sampling frequency decreased to alternate weeks from 1988 to 1991 during the fall. On alternate weeks, Project Quinte permanent stations in the upper (B, N), middle (HB), and lower bay (GL, C) were also sampled from 1988 to 1992 (for exact locations see Millard and Johnson 1986). PP data from the Bay of Quinte study (1988–1992) have been included in this paper to develop a relationship between seasonal PP and mean P levels. The Bay of Quinte data extended the gradient in P levels compared with the restricted range from only Lake Ontario. Consistent methodology between these projects eliminated a source of variability.

Temperature profiles were measured with an electronic bathythermograph and converted to density to determine mixing depth (Z_m). The water column was considered thermally stratified when a density gradient exceeded 0.08 $kg \cdot m^{-3} \cdot m^{-1}$ at a depth greater than 4 m, criteria similar to those used in other studies (Reynolds and Wiseman 1982; Reynolds et al. 1984; Millard 1986). Water samples were integrated from the surface to 20 m during isothermal or weakly stratified conditions and from the surface to the epilimnion depth minus 1 m under thermal stratification. Samples were placed in acid-washed, darkened, insulated 20-L polycarbonate bottles. Subsamples for measurement of nutrients, Chl, biomass, and photosynthesis were taken from the same bottle. Sample containers were cleaned with 10% sulphuric acid between cruises and rinsed thoroughly with distilled water then several times with sample water at collection time. Bay of Quinte stations were sampled from the surface to 2 times the Secchi disc depth as an approximation of the euphotic zone. Mixed depths in the Bay of Quinte usually exceeded the euphotic depth (Z_{eu} = 1% surface light penetration) except on rare occasions at station C. In Lake Ontario, Z_{eu} extended beyond the relatively shallow epilimnion (Z_m) in early summer. Samples from three equally spaced depths through this metalimnetic euphotic zone were pooled when this layer exceeded 3 m in thickness.

One-litre samples for Chl analysis were filtered through GF/C glass-fibre filters (Whatman Co.) and frozen for later analysis. Filters were ground and extracted in 90% acetone and analyzed spectrophotometrically for Chl (Strickland and Parsons 1968). Chl concentrations reported here are the mean of duplicate samples uncorrected for phaeopigments.

Solar radiation and light extinction

Light extinction of photosynthetically available radiation (PAR = 400–700 nm) was measured at 1-m intervals through the water column using an LI–192 underwater quantum sensor. An LI–190 quantum deck cell (LI-COR, Inc., Lincoln, Neb.) was used to check for variability of incident irradiance during the profile. Deck cell readings were used to normalize underwater readings taken on cloudy days. The extinction coefficient for PAR (ε_{par}) was calculated as the slope of a simple linear regression of the natural logarithm of light intensity versus depth. Mean epilimnetic irradiance ($\bar{I}$; $mE \cdot m^{-2} \cdot min^{-1}$; 1 $mE \cdot m^{-2} \cdot min^{-1}$ = 0.217 $W \cdot m^{-2}$) was calculated using the computer programs of Fee (1990) as

$$\bar{I} = \frac{I_s \int_0^{Z_m} I(z)dz}{Z_m} \quad (1)$$

where I_s is the mean daily (24-h average) solar irradiance at the lake surface, Z_m is the mixing depth, and $I(z)$ is the fraction of PAR reaching depth z. $\bar{I}$ was calculated using 30-min intervals throughout a 24-h time period using measured irradiance. $\bar{I}$ can be calculated with cloudless irradiance, which is best for showing the influence of Z_m and ε_{par} because it removes variability owing to cloud cover. We used $\bar{I}$ cal-

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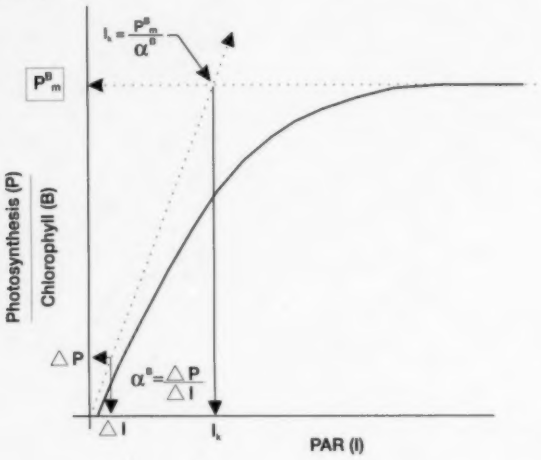
Fig. 2. Photosynthesis–irradiance relationship (solid line). The superscript B indicates that parameters are normalized on Chl concentration. The parameters are P^B_m, the maximal rate of photosynthesis; α^B, the slope of the light-limited part of the curve; and I_k (P^B_m/α^B), the level of PAR at the onset of light saturation and below which photosynthesis is light limited. After Fee et al. (1992).



culated with measured irradiance to analyze phytoplankton response to actual light conditions in the water column. $\bar{I}$ calculated with measured irradiance averaged 70.2 ± 22.2% of the cloudless values (n = 101). Long-term studies at the Experimental Lakes Area in northwestern Ontario have shown that the irradiance on an average cloudy day is 70% of the theoretical cloudless value (Fee et al. 1992). Our results for $\bar{I}$ and analysis of unpublished solar data indicate that the 70% value is also applicable to Lake Ontario (S. Millard, unpublished data).

Incident solar irradiance (PAR) was measured at both ends of the lake with LI–190 quantum sensors (LI-COR, Inc.). A sensor in the east end of the lake was mounted on top of the Point Petre, Ontario, lighthouse and 10-min integrals ($mol \cdot m^{-2}$) were recorded on a LI–1000 datalogger. The other sensor was mounted on the roof of the Canada Centre for Inland Waters (CCIW), Burlington, Ontario, and logged 30-min integrals to a Campbell 21X electronic integrator (1987–1989) and to a Campbell CR5 microprocessor datalogger from 1990 to the present. Sensors were calibrated yearly by the manufacturer. Daily integral photosynthesis rates at station 81 were calculated using solar data from Point Petre and solar data from CCIW were used for station 41 calculations.

Photosynthesis

Photsynthesis rates were determined by measuring ^{14}C uptake (Vollenweider 1974) of whole-water samples exposed to a light gradient in a shipboard incubator. The incubator was a rectangular trough made of 9-mm grey PVC (1.1× 0.4 × 0.15 m) with clear, 9-mm plexiglass at the end nearest the light source (Fee et al. 1989, 1992). The light source was a 150-W high-pressure sodium vapour lamp (Thorn Lighting, Mississauga, Ont.). A working tracer solution (approximate activity 18.5×10^5 $Bq \cdot mL^{-1} = 50$ $\mu Ci \cdot mL^{-1}$) was made by diluting stock $Na_2{}^{14}CO_3$ (Amersham Co.) with a carrier solution (unlabelled Na_2CO_3) of the same alkalinity as Lake Ontario. Five-millilitre aliquots of tracer solution were flame sealed in glass ampoules and autoclaved for later use. One litre of whole lake water was inoculated by dispensing the contents of one ampoule through an inline cellulose-acetate filter. The inoculated sample was well mixed and dispensed through a silicon tube to 10–15 light and 2 dark, 60-mL Pyrex bottles (Corning). Batch inoculation reduced interbottle variability. Bottles were incubated in a light gradient at close to in situ temperatures. Incubation periods were 3–4 h in the summer and 4–5 h in the spring when volume-based uptake rates were lowest. Metalimnetic samples were incubated in a separate incubator at the average temperature of the three depths sampled. Phytoplankton from this part of the water column were often shade adapted, and the optimal response was achieved using a light gradient spanning a lower range of intensities. The light level at all bottle positions was checked at least once during an incubation using a Biospherical QSP–200 scalar quantum sensor (Biospherical, San Diego, Calif., recalibrated yearly by the manufacturer). Light measurements at each bottle position were invariant over the incubation and replicate light measurements in the incubator were unnecessary. When bottles were removed at the end of an incubation they were replaced with a dummy set of filled bottles to ensure a consistent light environment for incubations not yet complete. Uptake at the end of the experiment was determined by the acidifying and bubbling technique (ABM) (Schindler et al. 1972). A 5-mL aliquot from each bottle was placed in a glass scintillation vial with 1 mL of 0.5 N HCl and bubbled for 30 min in a vacuum apparatus (Shearer et al. 1985). Five-millilitre standards were taken from three random bottles and placed in scintillation vials with 200 µL of ethanolamine to determine the total ^{14}C available for uptake. Samples were removed from the bubbler, capped, shaken, and left overnight with loose caps before 10 mL of Redi-Gel liquid scintillation fluor was added (Beckman Co., Mississauga, Ont.). Samples were counted in a Beckman LS5000 TD liquid scintillation counter for 20 min or to a 2 sigma level of accuracy of 2%. Quench corrections were made using the external standard technique and the $H^\#$ (Beckman Co.).

Photosynthesis calculations

Depth integrals of photosynthesis were calculated with the computer programs of Fee (1990). Chl concentrations, vertical profiles of transparency, incident solar irradiance, photosynthetic parameters, and mixing depth data are required as input to the programs. Percent transmission of PAR at depth was calculated from ε_{par}. The photosynthetic parameters P^B_m (the C uptake rate at light-saturating irradiance; mg C·mg $Chl^{-1} \cdot h^{-1}$), and α^B (the slope of the light-limiting part of the photosynthesis versus light intensity curve; mg C·mg $Chl^{-1} \cdot E^{-1} \cdot m^2$) were derived using the curve-fitting program PSPARMS (Fee 1990) and the photosynthesis versus light intensity data measured in the incubator (Fig. 2). The superscript B indicates that P^B_m and α^B are normalized per unit of biomass using Chl concentration uncorrected for phaeopigments as a biomass index. P_{opt} is the product of P^B_m and Chl; it is the unnormalized photosynthesis rate at optimal PAR.

Daily in situ photsynthesis rates were calculated with the programs YPHOTO and YTOTAL (Fee 1990). These programs calculate C fixed over longer periods of time by interpolation. Methods used in this study were identical to those described by Fee et al. (1989, 1992), making comparison with those data sets possible.

Long-term trends in PP

A comparison of our PP results with earlier studies (Glooschenko et al. 1974) and with those from the IFYGL in 1972 (Stadelmann et al. 1974; Stadelmann and Moore 1974) and the Lake Ontario Nutrient Assessment Study (LONAS) in 1982 (Lean et al. 1987*a*) was necessary to determine whether productivity has declined in response to P management. Long-term comparisons of photosynthesis can be hindered by methodological differences between studies (Shearer et al. 1987). Incubation methods and sampling frequency in these earlier studies differed significantly from those in our study. In addition, the earlier studies used the filtering technique and not ABM to determine the amount of tracer incorporated by the phytoplankton. Bowers et al. (1987) concluded that the method used to calculate PP was more important than whether incubator or in situ methodology was used.

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Table 1. Common statistics of relevant photosynthesis parameters, Chl concentration ($mg \cdot m^{-3}$), P_{opt} ($mg\ C \cdot m^{-3} \cdot h^{-1}$), P^B_m ($mg\ C \cdot mg\ Chl^{-1} \cdot h^{-1}$), α^B ($mg\ C \cdot mg\ Chl^{-1} \cdot E^{-1} \cdot m^2$), I_k ($\mu E \cdot m^{-2} \cdot s^{-1}$), and light extinction (ε_{par}; m^{-1}), for both stations over the study period (1987–1992).

	Mean	SD	Min	Max	*n*
Station 41					
Chl	3.40	1.47	1.10	9.24	157
P_{opt}	9.38	4.88	0.78	26.40	133
ε_{par}	0.30	0.09	0.17	0.59	143
P^B_m	2.86	1.31	0.36	7.53	133
α^B	4.24	2.02	0.64	13.45	133
I_k	198	72	59	444	133
Station 81					
Chl	4.03	1.17	1.25	7.48	159
P_{opt}	11.39	5.17	2.64	30.42	135
ε_{par}	0.35	0.08	0.19	0.74	146
P^B_m	2.86	1.22	0.50	7.43	135
α^B	4.39	1.75	0.96	11.69	135
I_k	186	62	79	381	135

We followed their suggestion and calculated PP from the IFYGL and LONAS data sets in a manner consistent with our study. The published IFYGL data and the raw LONAS data (LONAS data supplied by D. Lean, Environment Canada, Burlington, Ont.) were converted to a format compatible with the current version of the Fee programs. This was a more difficult task for the IFYGL data set because in situ profiles had to be converted to photosynthesis versus *I* curves to estimate the photosynthetic parameters required for the programs. The total irradiance recorded during the incubation period and ε_{par} were used to calculate average light intensity for specific incubation depths in the in situ profile. Surface points exhibiting photoinhibition were omitted. All of the required data were in Stadelmann and Moore (1974) and Stadelmann et al. (1974) for their main offshore station (Ontario Organic Particle Study, IFYGL station 19; Fig. 1). Light levels from IFYGL were converted from energy to quanta using an approximate conversion for daylight PAR of 1000 $erg \cdot s^{-1} \cdot cm^{-2}$ (= 4.6 $\mu E \cdot m^{-2} \cdot s^{-1}$; LI-COR, Inc.). Continuous records of solar irradiance measured in quanta were not available so seasonal PP was calculated using theoretical cloudless solar irradiance. Published estimates of integral PP during IFYGL were annual values but an estimate for our standardized season (May 1 – October 31) was made by setting appropriate start and end dates for YPHOTO and YTOTAL.

LONAS used a shipboard incubator to derive photosynthetic parameters; however, their incubator had only four light levels so there was greater potential for error in determining photosynthetic parameters than in our study where 10–14 points were used for curve fitting. Parameter estimates for LONAS were more easily biased by an aberrant data point or deletion of an outlier because only three points would have remained for curve fitting. Sampling cruises for both IFYGL and LONAS were much less frequent than in our study so the programs interpolated estimates of daily photosynthesis over longer time periods for seasonal estimates. LONAS sampling was monthly in the spring and every 2 weeks in July and August; only one cruise occurred in September–October.

Results

Determinant factors for integral photosynthesis

Light extinction

Mean ε_{par} at station 41 was slightly lower than at station 81 in each year and over the entire study period (Table 1). Mean ε_{par} equates to average euphotic depths of 15.4 and 13.2 m at stations 41 and 81, respectively. Mean ε_{par} prior to the average date of thermal stratification was slightly lower at both stations (0.30 m^{-1}) than the seasonal mean (Fig. 3). However, station 81 had high ε_{par} on the first spring sampling compared with the mean values in several years (1989, 1991, 1992). ε_{par} showed a distinct peak in late July to mid-August each year with the peak at station 81 often preceding that at station 41 by several weeks with the exception of 1988 and 1990 (Table 2). Maximum values for ε_{par} reduced Z_{eu} to 8–10 m when ε_{par} was 0.45–0.55 m^{-1} and to only 6 m at the maximum value recorded for ε_{par} over the study at station 81. Seasonal mean ε_{par} at stations 41 (0.27 m^{-1}) and 81 (0.31 m^{-1}) was lowest in 1992, about 10% below the overall mean.

Table 2. Peak light extinction (ε_{par}; m^{-1}), photosynthesis P_{opt} ($mg\ C \cdot m^{-3} \cdot h^{-1}$), and temperature (°C).

Year	Date	ε_{par}	P_{opt}	Temperature (°C)
Station 41				
1987	Aug. 19	0.59	21.5	23.5
1988	Aug. 14	0.52	12.5	26.0
1989	Aug. 11	0.47	15.7	19.2
1990	Aug. 30	0.45	12.6	21.4
1991	Aug. 29	0.55	15.9	22.1
1992	Aug. 19	0.47	26.4	17.3
Station 81				
1987	July 21	0.56	15.9	22.4
1988	July 27	0.74	21.0	23.1
1989	Aug. 1	0.55	22.8	21.9
1990	Aug. 28	0.48	12.9	22.7
1991	July 23	0.55	15.5	23.0
1992	July 28	0.48	30.4	23.0

Chlorophyll a *and* P_{opt}

The seasonal mean Chl concentration was similar at the two stations, but the overall study mean was slightly higher at station 81 with a lower coefficient of variation (CV = 29.0%) compared with station 41 (CV = 43.2%). Seasonal patterns differed between the two parts of the lake. Concentrations at station 81 were often highest early in the spring and declining when sampling started in mid- to late April, except in 1992 (Fig. 4). Declining diatom biomass resulting from nutrient limitation was likely the cause of this Chl trend (Millard et al. 1996). In contrast, spring values at station 41 started at lower levels and either increased through May and June to seasonal highs or stayed relatively high until late June (1989, 1992) (Fig. 5). The exception was 1992 when peak values were recorded in late July and early August at stations 81 and 41, respectively. Generally, early season trends in Chl reflected those for diatom biomass, which was controlled by different factors for each station (Millard et al. 1996). Both stations had high spring Chl in 1990 and 1991. This was less notable at station 81 because Chl was declining, whereas at station 41 the levels rose from low values in April to peak values by late May. A typical spring pattern for Chl was evident in 1988 although P_{opt} was atypically low until the end of June at both stations because of low P^B_m values.

Prior to stratification, mean P_{opt} was higher at station 41

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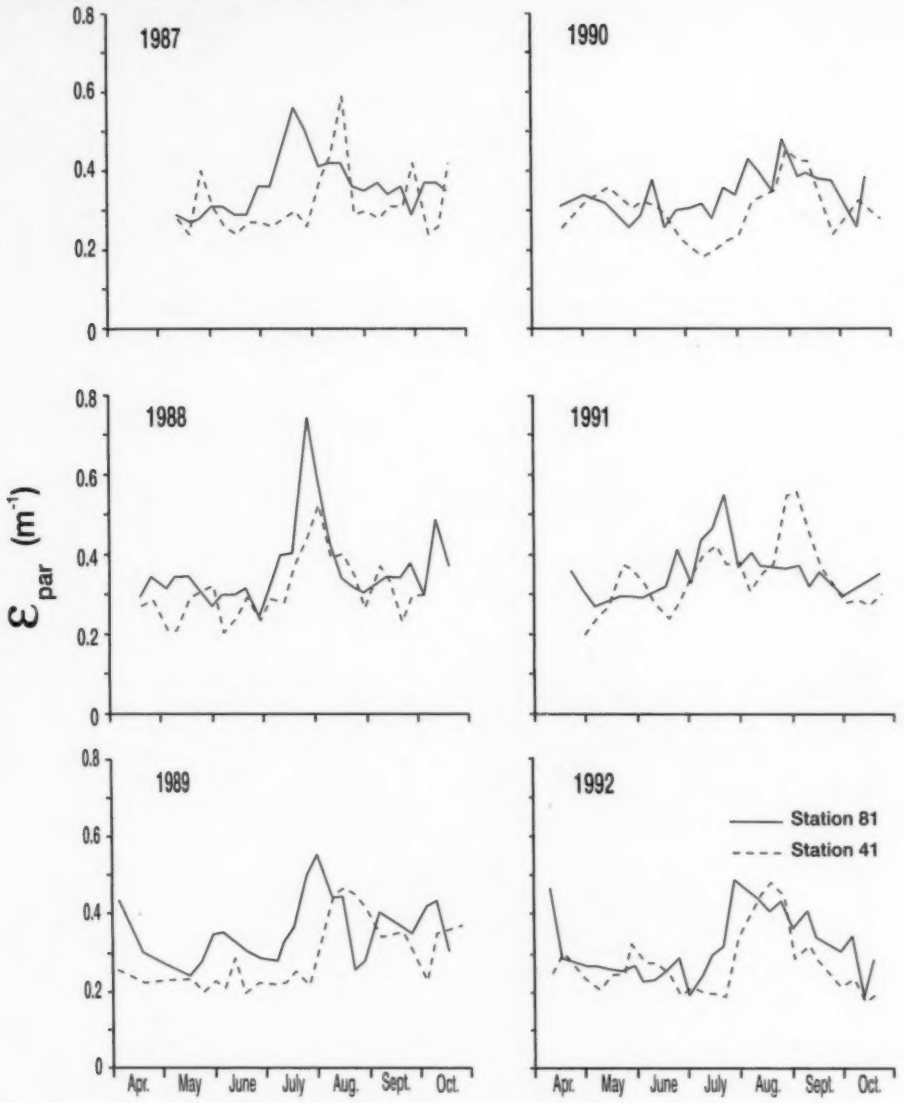
Fig. 3. Seasonal trends in ε_{par} at stations 81 and 41 from 1987 to 1992.



(8.40 ± 5.55 mg C·m^{-3}·h^{-1}) than at station 81 (6.74 ± 2.79 mg C·m^{-3}·h^{-1}), but seasonal means were higher at station 81 (11.39 ± 5.2 mg C·m^{-3}·h^{-1}) than at station 41 (9.38 ± 4.9 mg C·m^{-3}·h^{-1}). Spring peaks for P_{opt} (>15 mg C·m^{-3}·h^{-1}) were notable in certain years (1987, 1990, 1991) at station 41 and were associated with pronounced peaks in Chl and not P^{B}_{m}. Spring peaks in P_{opt} in these years were not evident at station 81.

A midsummer period of minimum Chl concentration occurred annually from early July to early August at station 41. These midsummer minima were followed by a rapid increase through August and into September in some years (1987, 1990, 1992), but there was a more gradual increase in others (1988, 1989, 1991). Summer Chl fluctuated less at station 81, but a midsummer decline was evident in 1991 and 1992. The mean Chl concentration for July at station 41 over the study was 2.11 ± 0.87 mg·m^{-3} but was much higher at station 81 (3.74 ± 1.49 mg·m^{-3}). P_{opt} peaked at station 81 by late July and at station 41 by early August, coincident with periods of lowest transparency. Mean summer (mid-July through August) P_{opt} were much higher at station 81 (16.1 ± 4.3 mg C·m^{-3}·h^{-1}) than at station 41 (11.5 ± 5.2 mg C·m^{-3}·h^{-1}). P_{opt} over 20 mg C·m^{-3}·h^{-1} was measured on 9 sampling days during the study at station 81, all in midsummer, but on only 3 days at station 41.

Chl did not increase appreciably at either station in the fall and P_{opt} declined at this time in all years (except 1992) coincident with the decline in photosynthetic capacity (P^{B}_{m}) and Chl. Chl and P^{B}_{m} fluctuated asynchronously from mid-August

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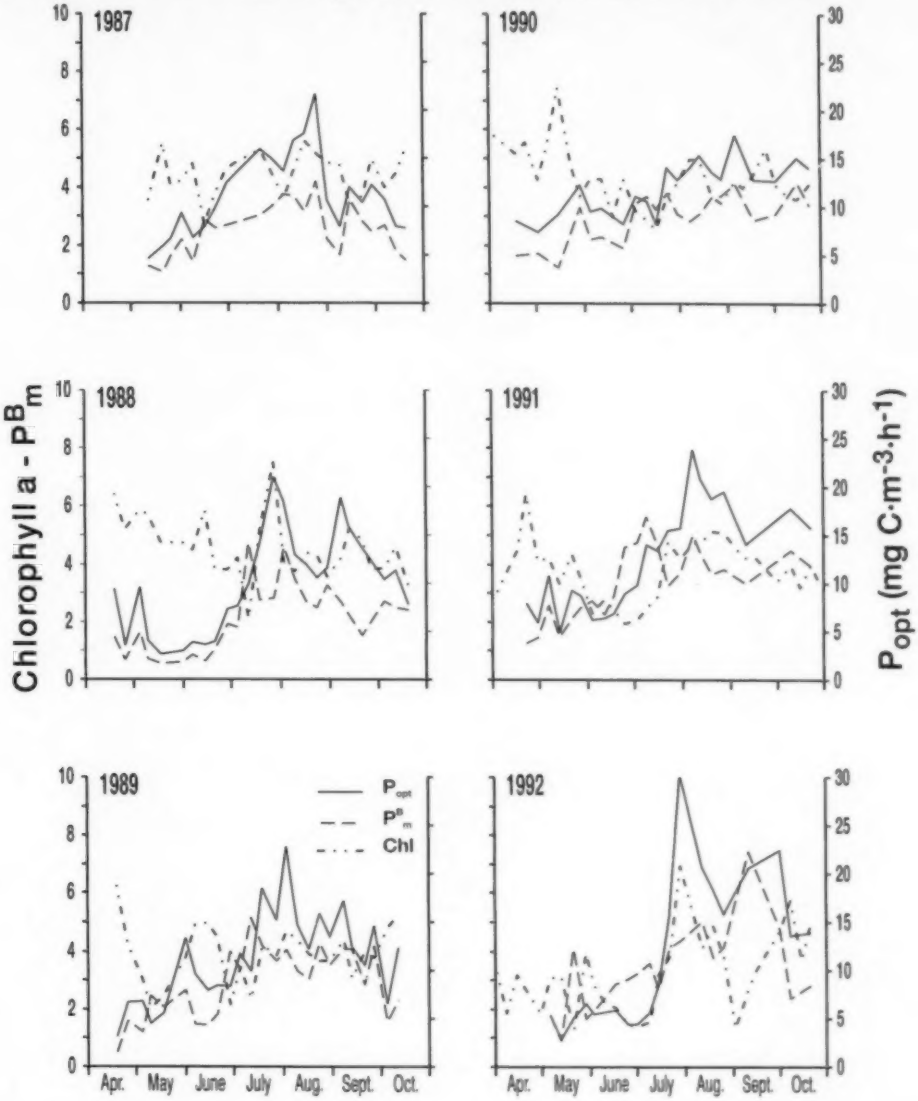
Fig. 4. Seasonal trends in Chl concentration, P^B_m, and P_{opt} ($P^B_m \times$ Chl concentration) at station 81 from 1987 to 1992.



through September in 1992. Peaks in P_{opt} at both stations occurred in the summer of 1992 owing to atypical increases in Chl. P_{opt} was maintained in late August and September by stable or increasing P^B_m as Chl declined.

Overall, P_{opt} was significantly related to Chl concentration at station 41 ($P_{opt} = 2.82 + 1.89$ Chl, $r = 0.58$, $n = 133$, $p < 0.01$), and the relationship improved if confined to sampling dates prior to thermal stratification ($r = 0.80$, $p < 0.01$). In contrast, at station 81 P_{opt} was not significantly related to Chl prior to thermal stratification but was significantly related ($P_{opt} = 4.00 + 2.17$ Chl, $r = 0.48$, $n = 104$, $p < 0.01$) following spring thermal stratification.

Seasonal means for P_{opt} and P^B_m in our study were not appreciably different from those measured during IFYGL (Table 3). Time-weighted seasonal means were used for the comparison to ensure that varying sampling frequency did not introduce bias. This was obviously not a factor in our study because these estimates did not differ from the arithmetic means in Table 1. The slight decline in P_{opt} (12.5%) appears to be due to a decline in Chl and not P^B_m. IFYGL Chl values were corrected for phaeopigments; however, uncorrected values would have been at least 20–30% higher on the basis of the average phaeopigment corrections from our study and others on Lake Ontario (Glooschenko et al. 1972). P^B_m would have also been proportionately lower if uncorrected Chl had been used. The low seasonal means for P^B_m and the effect on P_{opt} in the LONAS study are evident but Chl levels in this study were similar to those of the IFYGL data.

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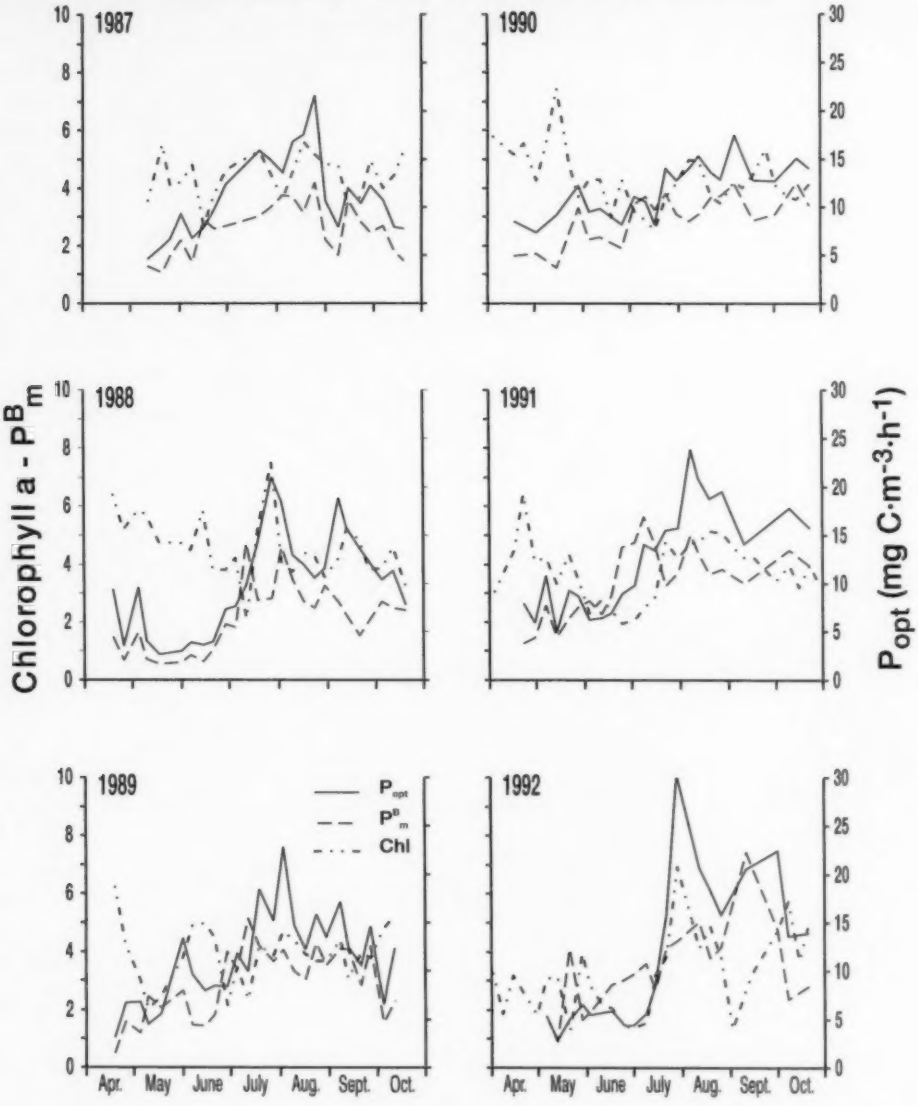
Fig. 5. Seasonal trends in Chl concentration, P^B_m, and P_{opt} ($P^B_m \times$ Chl concentration) at station 41 from 1987 to 1992.



Photosynthetic parameters: P^B_m *and* α^B

Mean P^B_m over the study was similar at both stations. Values were lowest in the spring, increased throughout the season until late summer, and either levelled off or declined in the fall (Figs. 6 and 7). Average P^B_m was the same at both stations during midsummer when Chl declined at station 41. P^B_m was positively correlated with temperature using all data from both stations ($P^B_m = 0.124$ temperature + 1.084, $r = 0.63$, $n = 261$, $p < 0.01$). Anomalously low values occurred in the spring of 1988 and persisted into late June when values increased to levels typical of other years.

Seasonal trends in P^B_m and Chl from station 41 were compared with those from IFYGL station 19 (Fig. 8). Means for station 41 were calculated for all data over the 6 years of the study during 2-week time windows starting in mid-April and running until the end of October for both variables. The number of observations within a window varied between 5 and 10 for April and fall windows and between 10 and 15 for those from May through August. Summer and fall Chl was higher in 1972 than in our study and would have been even higher if the IFYGL Chl data had been uncorrected for phaeopigments as in our study. Spring IFYGL Chl appears to be more variable than in our study but this may have been accentuated by the lower sampling frequency. P^B_m in our study remained consistently higher until September when the IFYGL study recorded much higher P^B_m values than our average value for this time window.

There were no well-defined seasonal trends or distinct differences between stations for α^B. There was a tendency for

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Table 3. Comparison of time-weighted seasonal means for P_{opt} (mg C·m^{-3}·h^{-1}), P^B_m (mg C·mg Chl^{-1}·h^{-1}), and Chl concentration (mg·m^{-3}) for stations 81 and 41 in 1987–1992, LONAS station 403 in 1982, and IFYGL station 19 in 1972.

Year	P_{opt}	P^B_m	Dates	Chl	Dates
			Station 81		
1987	11.54	2.60	May 12 – Oct. 20	4.47	*
1988	9.30	1.98	Apr. 19 – Oct. 20	4.59	*
1989	11.00	2.85	Apr. 5 – Oct. 18	3.83	*
1990	11.73	2.89	Apr. 18 – Oct. 24	4.34	Apr. 4 – Oct. 24
1991	13.21	3.50	Apr. 23 – Oct. 23	3.80	Apr. 4 – Oct. 29
1992	13.97	2.81	May 5 – Oct. 20	3.23	Apr. 1 – Oct. 20
Mean	11.79±1.65	2.77±0.49		4.04±0.52	
			Station 41		
1987	9.92	2.62	May 13 – Oct. 21	3.93	*
1988	6.75	2.14	Apr. 20 – Oct. 13	3.38	*
1989	8.75	2.97	Apr. 4 – Oct. 26	3.09	*
1990	10.53	2.99	Apr. 19 – Oct. 25	3.54	Apr. 3 – Oct. 25
1991	11.05	3.36	Apr. 24 – Oct. 24	3.23	Apr. 3 – Oct. 30
1992	11.49	3.46	May 7 – Oct. 22	3.39	Mar. 31 – Oct. 22
Mean	9.75±1.75	2.92±0.49		3.43±0.29	
			LONAS 403		
1982	5.04	1.32	Apr. 28 – Oct. 20	3.98	*
			IFYGL 19		
1972	11.14	2.81	Apr. 12 – Oct. 25	3.84	*

*Sampling period was the same as for the photosynthesis data.

values to be lowest in the spring and then increase in the late summer and fall in some years. In theory, phytoplankton could respond to changes in mean water column $\bar{I}$ by varying α^B, but a significant relationship with $\bar{I}$ was not present. The relationship between these two variables was still not significant when restricted only to the fall data. Weak relationships between α^B and light and lack of significant seasonal trends have been noted in other studies (Bindloss 1976; Fee et al. 1992). Values of α^B were usually maximal in the fall when $\bar{I}$ declined owing to deeper Z_m values and lower incident irradiance, suggesting that the phytoplankton were adapting to lower light levels. This response was not consistent; otherwise, a stronger relationship between α^B and $\bar{I}$ would have been observed. Increased photosynthetic efficiency (higher α^B) is a common adaptive mechanism for phytoplankton growing under lower light levels to increase C fixation (Steeman-Nielsen et al. 1962; Hecky and Guildford 1984). Spring values for α^B in 1988 were atypically low as noted for P^B_m. The high values for α^B in the spring of 1989 at both stations were not observed in other years. The relationship between α^B and temperature, although statistically significant, was very weak ($r = 0.26$, df = 1, 259), but the relationship with P^B_m was much stronger ($r = 0.69$, df = 1, 266).

The light saturation parameter I_k (P^B_m/α^B) defines the light requirements of the phytoplankton community for optimal rates of photosynthesis. Photosynthesis is assumed to be light limited when levels of light supply ($\bar{I}$) for the phytoplankton community fall below demand (I_k). I_k averaged 194 ± 70 μE·m^{-2}·s^{-1} for both stations and showed a seasonal trend similar to that for mean epilimnetic temperature, particularly at station 41. Values were lowest in the spring, increased during the summer, and declined again in the fall. The transition from low spring to higher summer I_k was usually due more to P^B_m increasing relative to α^B (because of the response of P^B_m to higher temperatures) than to α^B changing in response to the light environment. Summer values were usually highest at station 41, with 1990 an exception. I_k was atypically low at both stations in the summer of 1992 because of consistently high of α^B from late July through September. A significant positive relationship was found between I_k and temperature using all data from both stations ($p = 0.57$, df = 1, 221, $p = 0.01$).

Seasonal and daily photsynthesis rates

Daily areal rates of photosynthesis fluctuated markedly throughout the season at both stations, with a trend toward highest values in the spring and a decline throughout the summer to the fall noted in certain years (1987, 1989, 1990) (Fig. 9). In general, the seasonal patterns were similar at the two stations particularly in 1992. PP did not decline as distinctly as Chl in July at station 41, with the exception of 1992. P^B_m tended to be either increasing rapidly (1987, 1988, 1989) or close to maximum values (1990, 1991) when Chl declined in midsummer. The exception in 1992 occurred when peak P^B_m occurred in September, although July P^B_m values were typical of other years. Higher transparency also helped maintain midlake areal PP at levels similar to those at the east end in midsummer. The anomalously low values for both photosynthetic parameters during the spring and early summer of 1988 caused a notable decline in PP for this time period. For example, areal photosynthesis integrated over the May–June period for 1988 was only 35% of the mean for the other 5 years at station 41 and 56% of that at station 81. Mean summer (July–August) areal photsynthesis rates for station 81 (921 ± 377 mg C·m^{-2}·day^{-1}) and station 41 (722 ± 284 mg C·m^{-2}·day^{-1}) were slightly higher than July (610 mg C·m^{-2}·day^{-1}) and late summer (470 mg C·m^{-2}·day^{-1}) values reported for 1970 (Glooschenko et al. 1974). However, our summer estimates of daily photsynthesis rates were considerably lower than the estimates of 1156 and 1241 mg C·m^{-2}·day^{-1} in July of the IFYGL studies. Sampling frequency

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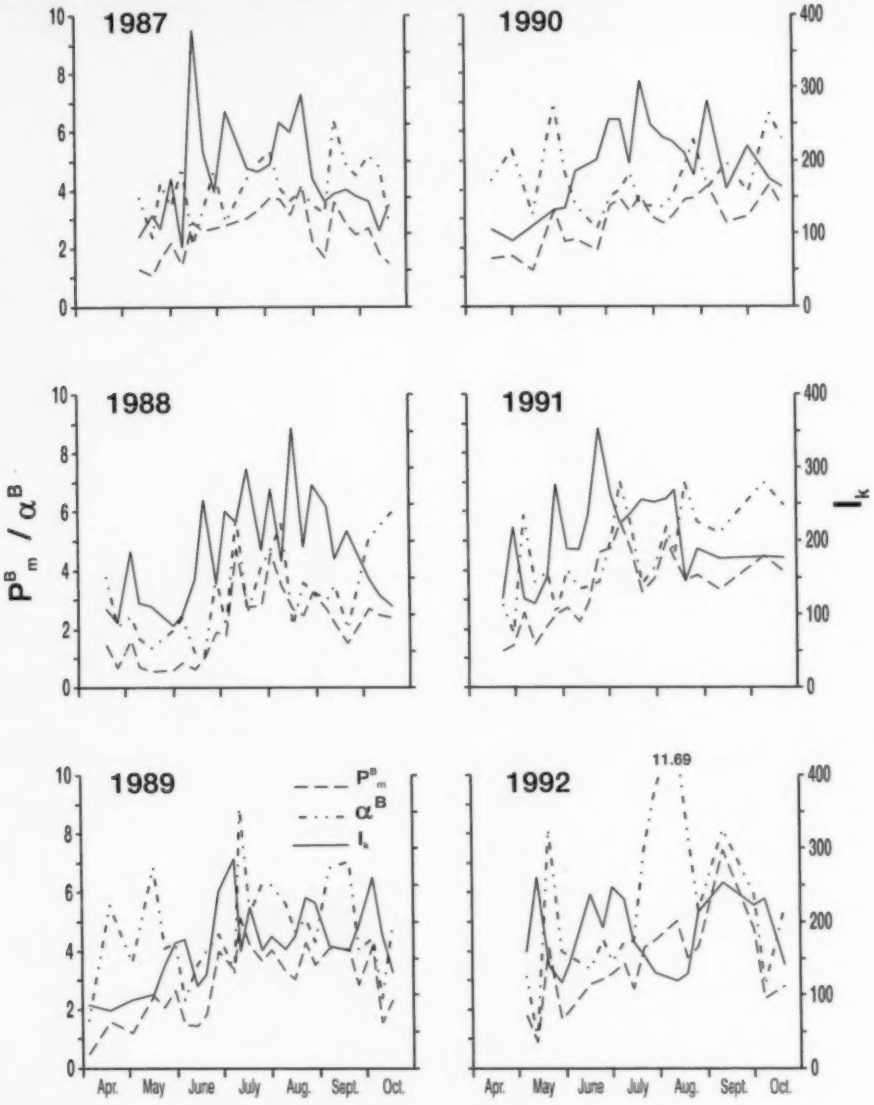
Fig. 6. Seasonal trends in P^B_m, α^B, and I_k (P^B_m/α^B) at station 81 from 1987 to 1992.



was much lower in these previous studies, which makes meaningful comparisons difficult.

Seasonal (May 1 – October 31) photosynthesis (g C·m^{-2}) was similar at both stations and showed remarkably low interannual variability with the exception of 1988 (Table 4). Seasonal PP measured at station 41 with empirical solar irradiance had a CV over the study of only 8% excluding the 1988 estimate and 17.8% including this estimate. Similarly, the CV for seasonal PP at station 81 was only 9% but increased to 14% with 1988 included.

There was a consistent relationship between PP estimated with empirical versus theoretical cloudless irradiance, suggesting that annual variability in solar irradiance was not an important factor for explaining annual differences in PP. The proportionality we measured between empirical and cloudless estimates of PP (76–78%) was very similar to that measured in other studies (Fee 1980; Hecky and Guildford 1984). We further tested the sensitivity of seasonal PP to annual variability in solar irradiance by running the 1991 data (Chl concentration, ϵ_{par}, P^B_m, α^B) in the photosynthesis programs with each of the solar irradiance files from 1987 to 1992. The CV for these six estimates of seasonal areal PP was only 2%. Millard and Johnson (1986) also demonstrated that over time frames of months, annual variability in solar irradiance was insignificant and they concluded that it had minimal impact on annual variability in PP. The estimates for 1987 for both stations may have been biased downward because sampling in the first year did not start until mid-May. Estimates for the earlier part of the

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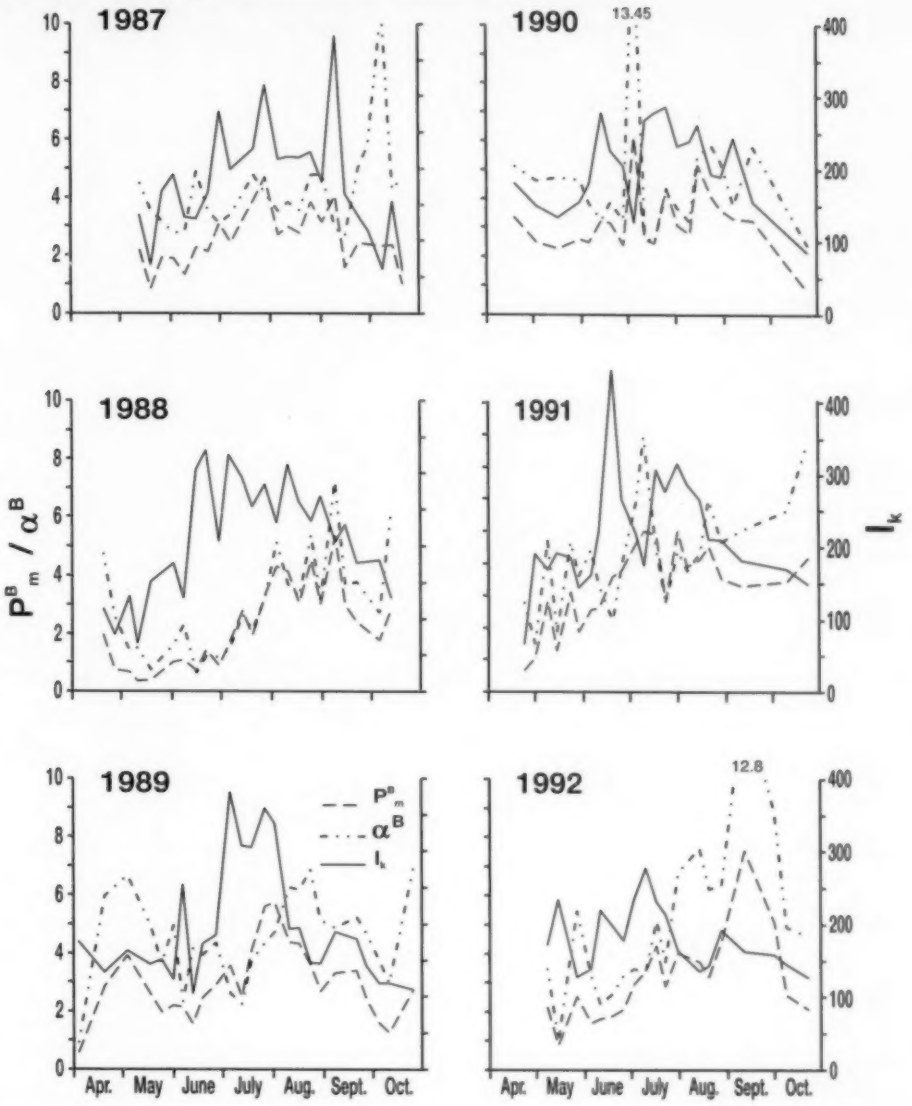
Fig. 7. Seasonal trends in P^B_m, α^B, and I_k (P^B_m/α^B) at station 41 from 1987 to 1992.



month were obtained by extrapolation with the photosynthesis programs.

Seasonal PP was distributed almost evenly between the seasons covered by our field season (Table 5). A notable difference between stations was that summer PP at station 81 was about 10% higher than in the spring or fall whereas fall PP at station 41 was about 7–9% lower than the values in the spring and summer. Yearly variability in the proportion of seasonal PP was lowest for the summer at both stations. Yearly variability was highest at station 41 in the spring and spring PP was higher than fall PP in some years. In 3 years (1987, 1989, 1990), spring PP at station 41 was 19–25% higher than in the fall. In comparison, spring PP at station 81 was over 10% higher than in the fall in only 1 year (13.2% in 1990).

Seasonal photosynthesis profiles showed that the eastern end of the lake was 15–20% more productive than midlake on a volume basis, similar to the difference in mean Chl levels (Fig. 10). However, the depth profile of photosynthesis did not penetrate as deep as in midlake because of lower average transparency but the higher P_{opt} values maintained areal rates at levels similar to the midlake. Seasonal P_{opt} for the IFYGL offshore station was similar to current levels for station 81 but much higher than for station 41. Higher rates were maintained throughout the water column for IFYGL than either of our stations, indicating that mean Chl concentrations and α^B values were higher. Seasonal volume-based rates for the LONAS stations were noticeably lower than for either station in our study, the result of lower estimates of P^B_m.

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Vertical distribution of photosynthesis

Depth profiles of photosynthesis extended into the metalimnion at both stations when stratification was relatively shallow and transparency high. These conditions occurred immediately following the onset of thermal stratification and extended into the early summer. Over the season this layer did not contribute significantly to water column photosynthesis, but on isolated days the subepilimnetic phytoplankton contributed significantly to the total depth integral of photosynthesis. On 33 of 157 experimental days at station 41 and on 15 days at station 81, this layer contributed 10% or more to the total areal photosynthesis rate. At station 41 these days usually occurred between mid-June and late July whereas at station 81 they were confined to a slightly earlier period from late May to early July. On 4 sampling days this layer accounted for 40–50% of total water column photosynthesis.

The subepilimnetic community had potential for rates of photosynthesis comparable to those of the epilimnion. However, in situ rates would never have approached the P_{opt} measured in the incubator because of subsaturating light intensities in this layer. Potentially high P_{opt} in the metalimnion was caused by similar or higher levels of Chl compared with those in the epilimnion, but lower P^B_m impaired photosynthetic potential (Table 6). Higher α^B in the metalimnion was likely the result of shade adaption of the phytoplankton community to the lower light intensities.

Calcium carbonate precipitation: whiting

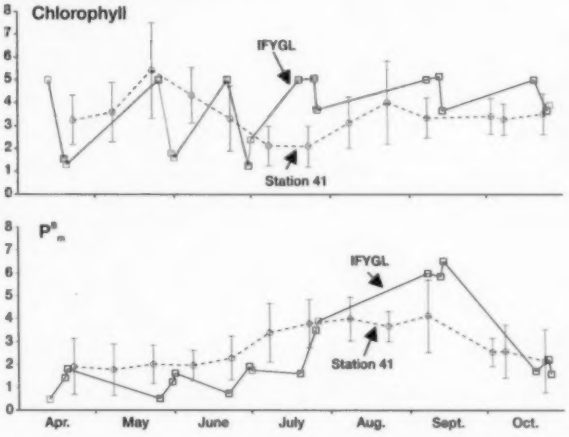
The annual summer increase in ε_{par} at both stations is caused by calcium carbonate (calcite) precipitation, referred to as whiting, a process common to lakes Ontario and Michigan (Strong and Eadie 1978). Decreased transparency owing to calcite precipitation has been observed in other studies (Koschell et al. 1983; Weidemann et al. 1985; Hanson et al. 1990). Lake Ontario is supersaturated with calcite and precipitation occurs when increasing temperature reduces solubility, and pH rises as a result of photosynthetic activity (Vanderploeg et al. 1987). The relative importance of temperature, photsynthesis rates, and Chl concentration to the seasonal trends in ε_{par} in Lake Ontario was tested using stepwise multiple regression. Seasonal peaks in ε_{par} coincided with maxima for P_{opt} at both stations in all years, explaining 41.5% of the variability in ε_{par} in a simple linear regression ($\varepsilon_{par} = 0.011\,P_{opt} + 0.213$, $r^2 = 0.415$, df = 1, 258, $p < 0.001$). Surface temperature and Chl both contributed significantly to the multiple regression, adding 7.2% to the total R^2 of 48.7%. The multiple regression equation relating ε_{par} to photsynthesis rates, temperature, and Chl concentration was

$$\varepsilon_{par} = 0.005(P_{opt}) + 0.022(\text{Chl}) + 0.006(\text{surface temperature}) + 0.115$$

$$R^2 = 48.7,\ F = 67.6,\ \text{df} = 3, 214,\ p < 0.001.$$

The influence of P_{opt} alone would have included a direct biomass effect because P_{opt} is the product of Chl and P^B_m. Chl contributed significantly to the regression after P_{opt} was entered, indicating that phytoplankton had a small but direct effect on ε_{par}. The effect may have been directly by light absorption or by the nucleation process for $CaCO_3$ precipitation (Koschel et al. 1983).

Fig. 8. Seasonal trends in P^B_m and Chl concentration from station 41 and IFYGL station 19. Points for station 41 are the means of all values falling within a 2-week time window from April to October 1987–1992. Error bars are ±1 SD.



Spatial differences in light extinction and their effect on seasonal photosynthesis

ε_{par} was consistently higher at station 81 than at station 41 over much of the season, although the difference was small (Table 1). The influence of this small difference in transparency on seasonal areal PP was tested by exchanging the transparency data between stations in the data files required as input to the photosynthesis programs. All other variables were left unaltered. Cloudless irradiance was used for calculations so that the influence of transparency alone could be assessed. The apparent minor difference in transparency had a significant impact on PP when integrated over a longer time scale, particularly at station 81 where PP increased an average of 19.3% with almost a 30% effect in 1 year when midlake transparency was used (Table 7). The negative influence of lower transparency on midlake results was not as drastic (11.9%).

Although P_{opt} was higher at station 81, resulting in part from higher Chl concentration, the small difference in seasonal mean Chl concentration could not likely have accounted for the difference in transparency based on average chlorophyll-specific extinction coefficients for PAR (Field and Effler 1983; Millard 1986). Higher Chl concentrations increase P_{opt}, but the potential increase in areal PP is offset by the self-shading effect, the negative influence that higher Chl concentration has on light penetration (Talling 1965; Millard and Sager 1994). If interstation differences in Chl concentration had explained the differences in transparency, then switching transparency data between the two stations would have interfered with the self-compensating mechanism, and the exercise would have been meaningless. We suggest that the seasonal difference in transparency and the effect on PP may be due to spatial differences in calcite precipitation or other abiotic particles that extend over a significant part of the season.

Long-term trends in phytoplankton photosynthesis

The only previously published values for annual PP in Lake Ontario are in the range 170–185 g C·m^{-2} (Glooschenko et al.

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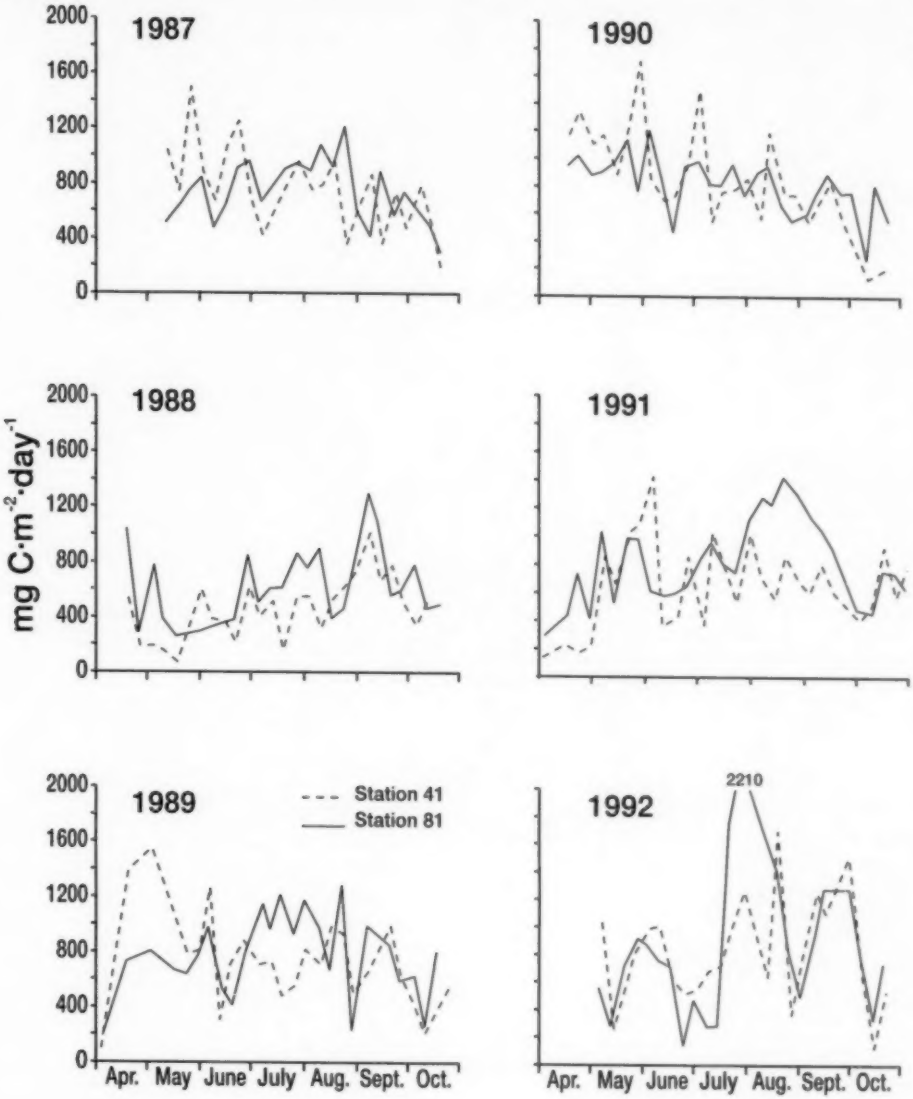
Fig. 9. Seasonal trends in daily integral phytoplankton photosynthesis at stations 81 and 41 for 1987–1992. Calculations were made with empirical solar irradiance.



1974; Stadelmann and Moore 1974; Stadelmann et al. 1974). Daily integral PP was measured very accurately in IFYGL experiments by summing the results from four to six short, in situ, sequential incubations made from dawn to dusk (Stadelmann et al. 1974). This method avoids extrapolation errors that occur when daily rates are determined from short-term incubations; however, results are still affected by the daily level of solar irradiance. Many of the IFYGL experiments were carried out on cloudy days and estimates of daily PP were biased downward as a result. Solar irradiance on IFYGL experimental days averaged only 43.4 ± 27.6% of theoretical cloudless levels with a range of 11–93%. The effect was magnified because daily rates of PP were extrapolated over relatively long time periods because of monthly sampling and annual PP was determined by integrating the area under the day rate versus time plot.

Recalculation of the in situ IFYGL data using the Fee programs made a substantial difference to some estimates of daily PP (Table 8) and to annual and seasonal PP (Table 9). Fitting P versus I curves to the in situ data did not alter P^B_m significantly but α^B values were slightly higher than those observed in our study and were unrealistically high in September. The estimate of annual PP for IFYGL using the reformatted data in the Fee programs and calculated with cloudless irradiance was 311 g C·m^{-2}. There was little variance in the relationship between seasonal PP values estimated using empirical irradiance

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Table 4. Seasonal (May 1 – Oct. 31) phytoplankton photosynthesis (g C·m^{-2}).

Year	Empirical	Cloudless	% of cloudless
	Station 41		
1987	133.8	172.7	77.5
1988	90.2	116.0	77.8
1989	136.0	184.8	73.6
1990	141.3	187.7	75.3
1991	142.4	179.0	79.6
1992	162.6	212.2	76.6
Mean	134.4	175.4	76.7±2.1
	Station 81		
1987	125.6	154.5	81.3
1988	108.2	137.8	78.5
1989	147.9	184.4	80.2
1990	144.0	189.2	76.1
1991	153.1	192.8	79.4
1992	161.1	208.9	77.5
Mean	140.0	176.3	78.8±1.9

Note: Empirical estimates use solar irradiance data collected at Point Petre for station 81 and at CCIW for station 41. Cloudless estimates use theoretical cloudless irradiance calculated using the programs of Fee (1990).

and cloudless irradiance in our study (mean = 78%; Table 4). It is unlikely that this relationship has changed significantly since 1972 because it is governed primarily by geographical climatic factors (Fee 1984). We scaled the annual cloudless PP estimate to 242 g C·m^{-2} for empirical irradiance by using the same proportionality of 78% from our study. Scaling to an estimate for empirical irradiance was necessary for comparison with the original published value. The recalculated estimate is considerably higher (43%) than the original published value of 170 g C·m^{-2} and shows how estimates of PP on cloudy days biased annual values. This new estimate may be better for long-term comparisons as calculations and extrapolation are consistent with our study and error owing to unusually low solar irradiance on sampling days has been removed. We also estimated seasonal photosynthesis with the reformatted IFYGL data for our standard season length at 245 g C·m^{-2} using cloudless irradiance and 191 g C·m^{-2} for empirical irradiance. These calculations indicate that seasonal PP may have declined almost 30% since 1972. Winter PP (November 1 – April 30) was estimated at 21% of the annual total for the IFYGL studies. If this winter proportionality is used to correct the published yearly value of 170 g C·m^{-2} for the offshore IFYGL station to our standard season, then seasonal PP in 1972 would have been only 134 g C·m^{-2} for the May 1 – October 31 period, very close to the estimates in our study. We would have concluded that PP had not changed since 1972 if the IFYGL results had not been recalculated.

The LONAS estimate for 1982 is about 44% lower than the average seasonal PP (1987–1992) for station 41, which suggests that the decline in PP since IFYGL has not been consistent. However, PP measured in 1982 may have been anomalously low for the early 1980s (similar to the 1988 data in our study). This is impossible to verify with data from only one season; however, the alternative approach described below allowed us to estimate an expected level of seasonal PP for 1982.

Seasonal phytoplankton photosynthesis and phosphorus

A relationship between seasonal integral PP and seasonal mean total P was developed using data spanning the trophic gradient from the eutrophic upper Bay of Quinte to Lake Ontario (Fig. 11). Least-squares regression was used to fit a function to the data (RS/1, BBN Software Corp., Cambridge, Mass.) of the same form ($y = ax/(b + x)$) as that used by Vollenweider et al. (1974) to relate annual PP to P loading for all of the Great Lakes. Parameterization of our curve fit was very similar to that obtained by these authors with the same upper limit of 420 g C·m^{-2}, although this was a seasonal, not annual, estimate in our study.

Data from 1988 are shown but not included in the model because spring PP appeared to be anomalously low. The regression predicts a seasonal PP value for the IFYGL of 206 g C·m^{-2}, close to the recalculated value. The predicted change in seasonal PP over the last 20 years based solely on the change in P concentration would be a decline from 206 g C·m^{-2} at 20 μg·L^{-1} in the offshore waters in 1972 to 136 g C·m^{-2} at a current total P level of about 10 μg·L^{-1}.

Lean et al. (1987*a*) estimated C fixation (June 7 – November 10, 1982) at 69.6 g C·m^{-2} (Table 2, in Lean et al. 1987*a*). Recalculation (raw data provided by D. Lean, Environment Canada, Burlington, Ont.) yielded a similar estimate of 75.4 g C·m^{-2} for our standardized season (May 1 – October 31). In contrast, the estimate of seasonal PP for LONAS station 403 using the regression of photosynthesis versus P and a seasonal mean P from nearby station 41 of 14 μg·L^{-1} (Johannsson et al. 1985) is 169 g C·m^{-2}, substantially higher than the published value.

Discussion

Long-term changes in productivity

It is difficult to conclude with certainty whether P control has reduced offshore phytoplankton productivity in Lake Ontario because there is neither a long-term data base nor previous multiyear studies of PP for comparison. Investigations of offshore PP in Lake Ontario have been few, the most recent being over a decade ago (Lean et al. 1987*a*). Furthermore, others worth noting have been for only one field season (Glooschenko et al. 1974; Stadelmann et al. 1974). Whether the IFYGL estimate of annual PP was typical of other years before P control was instituted is speculative. However, Glooschenko et al. (1974) arrived at a similar value for 1970, but calculation and extrapolation techniques for daily and seasonal photosynthesis in their study also differed significantly from our methods and were probably less accurate (Bowers et al. 1987). Other studies have characterized ecosystem productivity with seasonal or annual PP (g C·m^{-2}) and have shown a response to both decreases (Millard and Johnson 1986) and increases (Goldman 1981; Goldman and Amezaga 1984) in P loading. Reanalysis of the IFYGL data indicate that the productivity of Lake Ontario may have declined significantly (29%) in response to P control if 1972 was representative of other years before P control. We corrected estimates of annual areal PP from IFYGL for a bias that occurred because some in situ experiments were carried out under cloudy conditions. These low values biased the estimate of annual photosynthesis derived from the integration of the daily PP versus time plot

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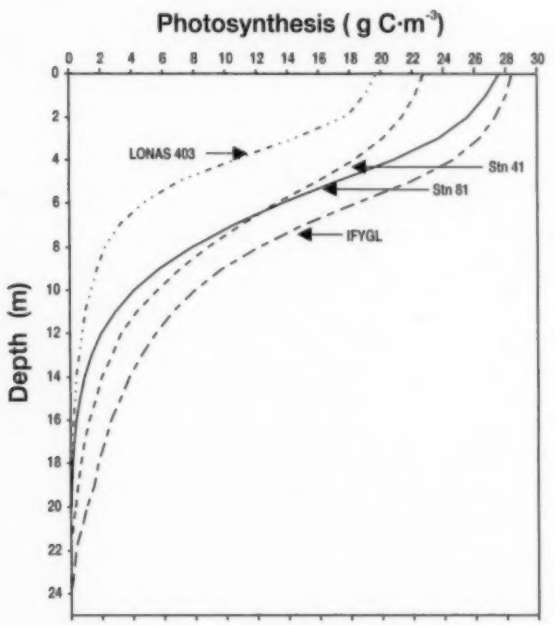
Table 5. Distribution (%) of total seasonal phytoplankton photosynthesis (g C·m^{-2}) for May 1 – Oct. 31.

	Spring		Summer		Fall	
Year	PP	%	PP	%	PP	%
			Station 41			
1987	60.5	45.2	43.0	32.1	30.3	22.6
1988	22.0	24.8	31.2	35.1	35.6	40.1
1989	56.5	41.8	47.8	35.4	30.8	22.8
1990	63.3	44.3	51.6	36.1	27.9	19.5
1991	52.7	37.3	47.0	33.3	41.5	29.4
1992	44.8	27.6	63.4	39.0	54.2	33.4
Mean	50.0±15.2	36.8±8.7	47.3±10.5	35.2±2.4	36.7±9.8	28.0±7.8
			Station 81			
1987	41.0	32.7	53.0	42.3	31.4	25.0
1988	26.4	24.5	42.7	39.6	38.6	35.8
1989	45.5	30.9	61.3	41.6	40.6	27.6
1990	55.4	38.7	51.4	35.9	36.5	25.5
1991	43.2	28.3	61.3	40.2	47.9	31.4
1992	36.8	25.4	54.1	37.3	54.1	30.4
Mean	41.4±9.6	30.1±5.3	54.0±7.0	39.5±2.5	41.5±8.2	30.4±5.3
			IFYGL*			
	41.9±2.3	21.6±1.0	85.1±2.2	44.1±1.4	66.3±2.9	34.3±1.2

Note: Photosynthetic rates were calculated with empirical solar irradiance. Spring, May–June; summer, July–August; fall, September–October.

*Average of separate calculations made with each solar radiation file, 1987–1992.

Fig. 10. Seasonal (May 1 – October 31) photosynthesis versus depth profiles averaged from 1987–1992 for Bioindex stations 81 and 41. Profiles for IFYGL station 19 and LONAS station 403 are also shown. Rates were calculated using theoretical cloudless irradiance for comparative purposes because continuous solar data were unavailable for LONAS and IFYGL.



because daily PP was interpolated on monthly intervals. The Fee programs linearly interpolate input variables (ε_{par}, Chl, P^B_m, α^B) for days between sampling dates but daily areal PP is calculated with measured solar irradiance data for that day. Consequently, interpolating poor ε_{par}, P^B_m, α^B, and Chl data can be an important source of error; however, daily variability caused by fluctuating solar irradiance is realistically modelled. Weekly sampling during much of our study undoubtedly reduced the potential for bad input data to bias seasonal estimates compared with both the IFYGL and LONAS studies, which had much lower sampling frequencies. Potential bias in calculation of seasonal PP that could occur if numerous solar data were missing was dampened over longer time periods because the programs use an average cloudy day (70% of cloudless irradiance) for missing solar data (Fee 1990).

The seasonal PP value predicted by the photosynthesis versus P relationship for the LONAS study was much higher than the empirical estimate, whereas the reestimated IFYGL value fell close to the fitted line. The agreement between seasonal PP predicted from P levels and the recalculated value helps confirm that the published IFYGL value was an underestimate. P^B_m values measured in LONAS were low compared with our study and may have contributed to lower than expected estimates of PP. Low P^B_m values persisted throughout the summer at station 403 with only two values in the 3.0–4.0 mg C·mg Chl^{-1}·h^{-1} range and 7 of 10 values from the study were <2.0 mg C·mg Chl^{-1}·h^{-1}. (D. Lean, Environment Canada, Burlington, Ont.). The comparatively low P^B_m values from LONAS are difficult to explain but may have been influenced by a slow rate of warming and late thermal stratification in 1982 (Rodgers 1987). The unusually low values in 1982 were similar to our findings in the spring of 1988. Other input data from LONAS (ε_{par}, Chl, α^B) were in the same range as those of our study. Various studies have suggested that ABM

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Table 6. Ratio of metalimnetic/epilimnetic photosynthetic parameters, P^B_m (mg C·mg Chl^{-1}·h^{-1}) and α^B (mg C·mg Chl^{-1}·E^{-1}·m^2), P_{opt} (mg C·m^{-3}·h^{-1}), and Chl concentration (mg·m^{-3}), 1987–1992.

	Mean	SD	Max	Min
		Station 41		
P_{opt}	0.91	0.47	2.54	0.41
P^B_m	0.71	0.24	1.60	0.24
α^B	1.50	0.60	3.55	0.40
I_k	0.60	0.25	1.00	0.22
Chl	1.32	0.58	3.13	0.48
		Station 81		
P_{opt}	0.85	0.27	1.70	0.39
P^B_m	0.82	0.23	1.37	0.52
α^B	1.43	0.50	2.89	0.86
I_k	0.70	0.27	1.21	0.35
Chl	1.07	0.27	1.74	0.55

Note: $n = 60$ for station 41; $n = 29$ for station 81.

Table 7. The effect of exchanging light extinction data between stations 81 and 41 on seasonal photosynthesis (g C·m^{-2}).

Year	Station 41			Station 81		
	Same ϵ_{par}	Exchange ϵ_{par}	% change	Same ϵ_{par}	Exchange ϵ_{par}	% change
1987	172.7	148.3	−14.1	154.5	193.5	25.2
1988	114.6	102.3	−10.7	137.8	158.7	15.2
1989	184.8	149.3	−19.2	184.4	238.4	29.3
1990	187.7	168.9	−10.0	189.2	223.7	18.2
1991	179.0	170.4	−4.8	192.8	210.2	8.3
1992	212.2	185.8	−12.4	208.9	259.5	19.5
Means	175.2	154.2	−11.9	177.9	214.0	19.3

Note: Values were calculated using theoretical cloudless irradiance.

yields higher values for ^{14}C uptake than the filtration method (Lignell 1982; Gächter et al. 1985; Søndergaard 1985). However, our use of ABM did not likely account for higher P^B_m values compared with LONAS, which used the filtering method. Early in our project, duplicate samples were analyzed using both ABM and filtration and a close relationship between the methods was found for P^B_m (ABM = 0.57 + 0.773 filter, $r = 0.84$, $n = 20$). The equation predicted similar P^B_m values for ABM compared with filtration in the range of our data (2–4 mg C·mg Chl^{-1}·h^{-1}). Conclusions on long-term trends can be seriously biased if based on a single season's data from years with anomalously low productivity such as 1982 or 1988. Results from single-year studies should be used cautiously in trend analysis, which reemphasizes the need for long-term studies and consistent methodology.

Summer and fall PP in our study were lower than during IFYGL. The mean PP for the July–August period during our study at station 41 was 47.3 g C·m^{-2} compared with 85.1 g C·m^{-2} estimated for the same months in 1972. August PP in IFYGL may have been biased upward because of high P^B_m estimated for August. August values were all determined in the Fee programs by interpolating between a value of 3.8 mg C·mg Chl^{-1}·h^{-1} in late July and values of over 6.5 mg C·mg Chl^{-1}·h^{-1} in early September. Infrequent sampling during August and September in IFYGL was a definite deficiency.

Table 8. Results of reformatting IFYGL in situ data to photosynthesis versus irradiance curves and calculating daily phytoplankton photosynthesis (PP-F; mg C·m^{-2}·day^{-1}) using the Fee photosynthesis programs in comparison with original estimates (PP-I; mg C·m^{-2}·day^{-1}).

Date	PP-I	PP-F	P_{opt}*	P^B_m-I†	P^B_m-F	α^B	Chl‡
Apr. 18	379	438	2.41	1.55	1.42	3.13	1.55
Apr. 19	273	524	2.43	1.87	1.81	8.88	1.30
May 30	278	518	2.30	1.28	1.25	3.89	1.80
May 31	470	605	2.70	1.69	1.63	3.75	1.60
June 29	446	649	2.74	2.19	1.92	8.0	1.25
June 30	468	1092	4.24	1.77	1.75	6.44	2.40
July 25	1241	1826	18.0	3.56	3.50	4.82	5.05
July 26	1156	1383	14.0	3.78	3.89	5.30	3.70
Sept. 12	1443	1875	33.3	6.47	5.84	14.65	5.15
Sept. 13	893	1495	24.5	6.71	6.52	30.67	3.65
Oct. 24	262	984	8.16	2.24	2.22	10.68	3.65
Oct. 25	344	783	6.17	1.58	1.58	4.72	3.90
Nov. 28	99	303	2.95	2.11	2.07	2.93	1.40
Jan. 19	58	145	1.14	1.34	0.26	6.47	0.85
Mar. 15	194	269	2.84	2.27	2.00	5.96	1.25
Mar. 16	237	295	2.22	1.64	1.45	5.00	1.35

Note: P^B_m-F (mg C·mg Chl^{-1}·h^{-1}) and α^B (mg C·mg Chl^{-1}·E^{-1}·m^2) are the photosynthetic parameters derived from the IFYGL in situ data after reformatting.

*Values are given as mg C·m^{-3}·h^{-1}. Original data from Stadelmann and Moore (1974).

†Determined from P_{opt}/Chl.

‡ Areal Chl (mg·m^{-2}) from Stadelmann et al. (1974) was divided by 20 to convert values to milligrams per cubic metre.

Schelske and Hodell (1991) suggested that up to 1987, summer PP had not declined in response to P controls. Their conclusion agreed with the anomaly discussed by Stevens and Neilson (1987) that although spring P levels had declined until 1982, summer values had not. These studies covered time periods that ended before our study started. A clear trend toward declining summer P has now emerged on the basis of data covering a time period ending in 1992 (Johengen et al. 1994). Our conclusions are also based on direct measurements of C fixation starting at the time the core of Schelske and Hodell (1991) stops and continuing to the present. Stevens and Neilson (1987) correctly pointed out that researchers in the early 1980s were trying to measure a response to a relatively small change in P concentration when other factors such as climate and food-chain interactions may have also played a role in masking a clear response to declining P. Furthermore, conclusions may have been hasty, as a time frame longer than the hydraulic residence time of the lake (6 years) was probably needed to ensure a productivity response. Low interannual variability of PP in recent years (1988 excepted) suggests that climate rather than P may now contribute more to yearly differences because there has been minimal change in P concentrations since the late 1980s. The low interannual variability in our study supports the suggestion by Fee et al. (1992) that increasing lake size has an influence in reducing yearly variability in photsynthesis rates.

Fee et al. (1992) suggested that the perceived lack of response in Lake Ontario has been due to differences in the underwater light climate compared with smaller lakes that tend

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Table 9. Comparison of seasonal (May 1 – Oct. 31) estimates of phytoplankton photosynthesis with previous studies.

Study	Station	Published PP (g C·m^{-2})	Annual*		Seasonal	
			Cloudless	Empirical	Cloudless	Empirical
IFYGL	19	170–185[†]	311	242	245	191
LONAS	403	70[‡]			96	75
Bioindex[§]	81				176	140
	41				175	134

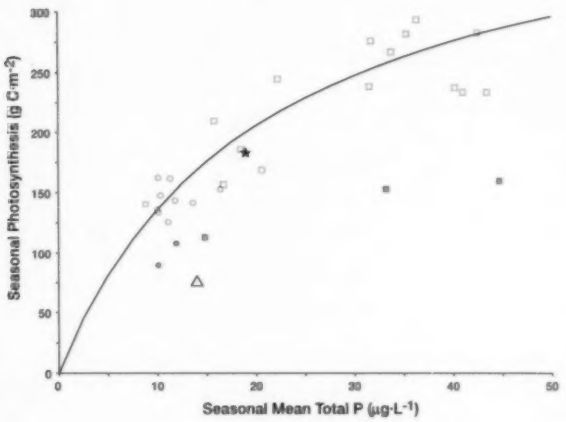
*Calculated using the Fee programs for April 1972 to March 1973. IFYGL and LONAS values were calculated with cloudless irradiance only. Empirical estimates use the average proportionality between cloudless and empirical estimates in Table 4.

[†]Annual estimate, April 1972 to March 1973, from Stadelmann et al. (1974).

[‡]Data for June 7 to November 10, 1982, from Lean et al. (1987*a*).

[§]Mean for 1987–1992. See Table 4 for annual values.

Fig. 11. Relationship between seasonal (May 1 – October 31) integral phytoplankton photosynthesis (PP) and seasonal mean total phosphorus (TP) concentration (PP = 419.8TP/(20.83 + TP), $r^2 = 0.81$, $n = 27$). The circles represent Lake Ontario data from this study and the squares represent data from the Bay of Quinte. Each point represents an entire season of study at a location. The five solid points are 1988 data excluded from the relationship. The recalculated IFYGL (star) and LONAS (triangle) estimates are also shown.



to be more nutrient deficient. However, they based their conclusions largely on the earlier LONAS study. These authors suggested that the mixed layer is almost always light limited in larger lakes, but this is consistently true for Lake Ontario only when the mixed depth exceeds about 38 m (Millard et al. 1996). P is limited for at least 3 months during the summer even in the deeper parts of the lake (Lean et al. 1987*b*; Millard et al. 1996). The shallow Kingston basin is P and Si limited for a much longer period than the offshore regions, which represent much of the lake surface area. Lower PP during these P-limited parts of the season as P levels declined was a realistic expectation.

The subepilimnetic layer did not contribute significantly to seasonal areal photosynthesis rates but was important on some dates in early summer. The photosynthetic components of this deeper euphotic layer indicated photosynthetic potential but in situ rates were limited by light penetration. Shade adaption by the phytoplankton in this layer to lower light levels was evident in lower I_k and higher α^B, but lower P^B_m than in the epilimnion indicated lower photosynthetic potential owing to either lower temperature or poor physiological health of sedimenting cells. This layer is unlikely to become more important to seasonal integral photosynthesis unless transparency increases. However, in Lake Michigan the subepilimnetic layer is a more important fraction of summer integral PP than in Lake Ontario, averaging 50% of the total areal rates during the summer with a range of 32–92% (Fahnenstiel and Scavia 1987). Cascading trophic interactions from the top of the food chain led to deeper photosynthesis in Lake Michigan when higher grazing pressure on phytoplankton increased transparency. It is conceivable that extensive zebra mussel (*Dreissena polymorpha*) colonization in Lake Ontario could cause a similar transparency change and increase the importance of photosynthesis in deeper layers.

P^B_m is an important physiological property of the phytoplankton community because it shows the photosynthetic response under nonlimiting light conditions to other environmental factors such as temperature and nutrients (Lehman 1976; Senft 1978; Glover 1980). The regression parameters in the relationship between P^B_m and temperature have not changed in over 20 years (Stadelmann et al. 1974) in spite of changes in phytoplankton species composition and nutrient levels (Stevens and Neilson 1987; Gray 1987). More recently, Makarewicz (1991) reported a similar relationship between P^B_m and temperature for two nearshore stations in Lake Ontario but accounted for only 25% of the variability in P^B_m with temperature compared with 40% in our study. The consistent temperature response suggests that P^B_m is a conservative property of the phytoplankton community in Lake Ontario, but anomalous responses can occur such as during the spring of 1988. Our findings support the statement by Harris and Piccinin (1977) that algae may have a universal temperature response for photosynthesis.

P^B_m in our study was higher than in Lake Michigan where values did not change between 1972 (Fee 1973) and 1984 (Fahnenstiel and Scavia 1987). Higher P^B_m in Lake Ontario has likely been caused by higher temperatures, although higher P levels cannot be ruled out (Curl and Small 1965). We noted a pronounced rise in P^B_m as water temperatures increased from spring to summer, particularly in the eastern end of the lake, although other studies in both Lake Michigan (Fahnenstiel et al. 1989) and Lake Ontario (Makarewicz 1991) have re-

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ported inconsistent seasonal trends in P^B_m. P^B_m values from our study were similar to IFYGL values (assimilation numbers; Stadelmann et al. 1974), but IFYGL estimates used Chl concentrations corrected for phaeopigments. Average phaeopigment corrections in our data set were 26.6 ± 17.1% and would have increased our P^B_m estimates by this proportion if they had been used in the photosynthesis–irradiance curve fitting. This would have made our spring values consistently higher and would have brought our late summer values more in line with IFYGL estimates. Glooschenko et al. (1974) reported P^B_m values for Lake Ontario during the 1970s on the basis of uncorrected Chl concentrations that were much lower than our values. Long-term changes in photosynthetic C fixation in both lakes Ontario and Michigan appear to be due primarily to changes in Chl concentration (Kwiatkwoski 1982; Johengen et al. 1994) and not photosynthetic properties of the community. Calculations of areal PP with the Fee programs are unaffected by choice of corrected versus uncorrected Chl concentration because changes in Chl levels are offset by proportional changes in photosynthetic parameters during curve-fitting of the P versus I relationship and the calculated in situ photosynthetic rates are the product of the parameters and Chl concentration.

The lack of seasonal trend in α^B and the insensitivity to temperature have been noted in other studies of PP (Bindloss 1976; Fee et al. 1992). The absence of a temperature response of α^B was not unexpected given that α^B is essentially a photochemical reaction and not an enzymatically controlled process (Harris 1978). Our results support the conclusion of Fee et al. (1992) that the significant correlation of P^B_m and α^B was probably due to normalization of both variables on the common parameter Chl as seasonal patterns between the parameters themselves were not closely linked. The temperature dependence of I_k was mediated through the effect of temperature on P^B_m, which caused most of the seasonal variability in I_k and led to maximal values in the summer (Bindloss 1976). Lower P^B_m and some higher α^B values in the fall were likely an adaptive community response to deep mixing and lower temperatures, although the α^B response to lower I was not consistent.

Photosynthetic productivity is optimized in lakes because phytoplankton communities adapt to changing conditions with compensatory physiological (P^B_m, α^B) and population (species succession) responses. PP did not decline proportionally to the July minimum in Chl concentration during the period of severe P limitation at station 41 (Millard et al. 1996). The July phytoplankton community is dominated by a mixed assemblage of phytoflagellates and green algae (Johannsson et al. 1985; O.E. Johannsson, unpublished data). Succession from a spring diatom community to nanoplankton that typically have higher P^B_m (Rodhe et al. 1958; Findenegg 1965; Pollingher and Berman 1982; Munawar et al. 1987) compensated for the decline in Chl concentration and led to a less than proportional drop in areal rates of photosynthesis. There was no distinct summer decline in Chl levels during IFYGL at the midlake station (Munawar et al. 1974), suggesting that phytoplankton may have been less P deficient during the summer prior to P control.

The unusually low P^B_m values during the spring and early summer of 1988 caused low estimates of areal PP compared with other years. All possible methodological influences were examined and ruled out as a cause for the anomalous results. Climatic variables, water temperatures, solar irradiance, stratification patterns, and nutrient levels have all been examined as explanatory factors, but spring conditions in these variables were not atypical compared with other years in the study. Only spring diatom biomass was significantly different in 1988 from that in other years. Biomass was less than half of the mean levels observed from 1987 to 1991, suggesting that P^B_m may have been responding to either P or Si deficiency that was limiting diatom growth.

Whiting and the potential effect on areal photosynthesis

The sensitivity of seasonal areal PP to small changes in ε_{par} was demonstrated by switching transparency data between stations 81 and 41. The small difference in mean light extinction between the two stations equated to only a 2-m difference in average euphotic depth, but there was a notable effect on areal PP when this difference was extended over the season. Global warming could potentially alter areal PP because epilimnetic temperatures and the length of the stratified period are likely to increase (McCormick 1990). Consequently, water clarity would decrease because whiting increases with temperature (Strong and Eadie 1978), and areal PP could theoretically be reduced. P_{opt} and temperature were both significant determinant variables in the multiple regression with ε_{par}, although most of the variability was explained by P_{opt}, similar to the findings for Lake Michigan (Vanderploeg et al. 1987). Future epilimnetic warming could increase ε_{par} directly by reducing calcite solubility and indirectly through an increase in P_{opt} ($P^B_m \times$ Chl concentration) as P^B_m has been shown to increase with epilimnetic temperature. If ε_{par} increases primarily because P_{opt} increases with P^B_m then the potentially lower areal PP should be partially offset by the positive effect of P_{opt} on areal rates because the photosynthesis–depth profile is expanded at shallow depths where light is not limiting. This is an interesting contrast with the self-shading described previously where the compensating response of areal PP occurs as P_{opt} fluctuates with Chl concentration, not P^B_m (Talling 1965; Millard 1986). Hecky and Guildford (1984) pointed out that integral primary production is relatively insensitive to ecosystem change because of compensatory responses in component variables. Nevertheless, there is the potential for future changes in climate and P loading to affect PP and the determinant variables P^B_m and ε_{par}.

Phytoplankton photosynthesis, phosphorus, and fisheries management

Our study indicates that primary productivity may have declined by 30% in Lake Ontario between 1972 and 1987–1992 coincident with the decline in P concentrations in the lake. If this reduction in productivity is transferred higher in the food chain, fisheries managers may have to mould public expectations toward lower fish production. Resource user groups differ on whether or not reduced lake productivity constitutes a beneficial outcome of P management. Certainly, lower *Cladophora* productivity in the nearshore (Painter and Kamaitas 1987; Painter and Jackson 1989) has been a positive outcome of P control. On the other hand, fisheries and P management goals for Lake Ontario may conflict if lower lake productivity is responsible for a decline in the numbers and size of prized sport fish (Hartig et al. 1991; Siemer and Brown 1994).

The lakewide management plan (LaMP) for Lake Ontario required under the Great Lakes Water Quality Agreement

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should provide a mechanism by which these conflicts can be resolved to meet societal needs. There has been recent debate whether municipal P controls in the lower Great Lakes should be relaxed to increase offshore P supply with the expectation that phytoplankton and consequently fisheries productivity will respond proportionately. However, hard evidence to support whether desired fish populations would respond positively to this action is lacking. The LaMP must weigh narrowly focused benefits against broader implications to society such as the negative impact higher P has on nearshore *Cladophora* growth, shoreline aesthetics, and provision of potable water. Society at large would likely view a relaxing of P control in the lower lakes as regressive ecosystem management. The future challenge to fisheries management agencies on both lakes Ontario and Erie will be to provide a sustainable sport fishery to the angling public, concurrent with declining lake productivity.

The importance of P loading to fisheries productivity depends on the importance of P relative to other phytoplankton growth factors such as light (Lean et al. 1987*b*; Millard et al. 1996), Si (Schelske et al. 1986), and energy transfer to the top of the food chain (Borgmann 1982; Flint 1986; Mazumder et al. 1992). Understanding the relative effect of these other factors is key to resolving to what extent fisheries and water-quality goals conflict. Our study has already contributed to an informed management decision that reduced salmonid stocking in 1992 (Lake Ontario Committee 1992; Bowlby et al. 1993) and will be invaluable in future modelling efforts relating primary and fish production (McConnell et al. 1977; Oglesby 1977; Downing et al. 1990; Borgmann 1982; Borgmann et al. 1984).

The generality of the seasonal photosynthesis–P relationship (Fig. 11) should be tested with data from a variety of lakes differing in trophic status and geographic location. However, the relationship may be useful now in determining the impact of zebra mussel invasion on phytoplankton productivity in the lower Great Lakes. Measured seasonal PP can be compared with the potential set by P because the relationship was developed in P-limited systems relatively free of zebra mussel effects. The relationship may be useful for lake management if further reductions in P loading are contemplated. Areal PP drives the productivity of the pelagic food chain from the bottom up. This process will be more sensitive in the future to small reductions in P because concentrations are now close to the linear portion of the photosynthesis–P relationship ($<10\ \mu g \cdot L^{-1}$). The linear response of photosynthesis at low P levels and the rapid constraint this could place on forage base production should be considered if further reductions in P loading are proposed.

Predictive models of ecosystem response to environmental perturbations (seasonal areal PP vs. P) including climate change, increased nutrient loading, or changes in food-chain structure can only be developed through long-term monitoring of ecosystems. Periodic single-season studies are of limited value in assessing long-term trends but have provided important information on shorter term physiological processes in Lake Ontario (Cuhel and Lean 1987; Lean and Cuhel 1987). We have shown that conclusions on long-term changes in productivity can be influenced by methodology. Consequently, long-term studies (>5 years) of ecosystem productivity require consistent and precise methodology throughout the study (Millard and Johnson 1986; Shearer et al. 1987). Our extensive data base will provide future researchers and monitoring programs with the information to determine unambiguously whether productivity of the lake has changed.

Acknowledgments

The success of this project would have been impossible without the help of the following students and technical staff: Warren Norwood, Suzanne Brett, Leanne Gris, Vicki McKay, Christine Burrows, Bill Gardner, and Brian Giles. Special thanks to the officers and crew of the CSS *Bayfield* and *Lauzier* for meeting rigorous scheduling demands and safely piloting us through rough conditions. Staff of the Technical Operations section, Environment Canada, provided assistance in the field. Special thanks to Everett Fee for assistance with incubator methodology and the photosynthesis programs at the start of the study and for critically reviewing the manuscript. Dave Lean also reviewed the manuscript and unselfishly provided the raw photosynthesis data from LONAS. Critical reviews were also provided by Ken Minns and two anonymous reviewers. Thanks to Jim Moore for writing computer programs that simplified data handling. Hugh Jones of the Canadian Coast Guard provided access to the Point Petre lighthouse for datalogger installation.

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Seasonal phosphorus deficiency of Lake Ontario phytoplankton at two index stations: light versus phosphorus limitation of growth

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Abstract: Seasonal patterns in the phosphate deficiency index and orthophosphate turnover time and factors determining the mean water column irradiance ($\bar{I}$) such as vertical mixing and light attenuation were measured at two locations in Lake Ontario in 1988 and 1989. The seasonal transition from light to phosphorus limitation was driven by the change in vertical mixing although this was most pronounced in midlake. Different trends in spring diatom biomass and nutrient deficiency between the two stations were caused by different mixing depths and the effect on the underwater light climate. Prior to thermal stratification, phytoplankton growth in the eastern basin was phosphorus- and silica-limited while light was the limiting factor in the midlake until stratification. $\bar{I}_{lim}$, the level of water column irradiance determining whether light was limiting phytoplankton growth, was estimated at 4–6 $mE \cdot m^{-2} \cdot min^{-1}$. $\bar{I}_{lim}$ occurred at a mixing depth of 38–50 m at the seasonal mean light attenuation of 0.30 m^{-1} under cloudless conditions on June 21. Light-limiting ratios, the ratio of light supply ($\bar{I}$) to light demand as defined by I_k (irradiance defining the onset of light saturation of photosynthesis), were compared with seasonal trends in the phosphate deficiency index.

Résumé : En 1988 et 1989, des chercheurs ont mesuré à deux stations du Lac Ontario les variations saisonnières de l'indice de carence en phosphate, le temps de renouvellement de l'orthophosphate et les facteurs qui déterminent le degré moyen d'éclairement énergétique ($\bar{I}$) dans la colonne d'eau, comme le brassage vertical et l'extinction de la lumière. Le passage saisonnier de la lumière au phosphore comme facteur limitant était induit par un changement dans le brassage vertical; ce phénomène était cependant le plus prononcé dans le milieu du lac. Les différences entre les tendances printanières que prend la biomasse des diatomées ainsi que les variations de la carence en nutriments observées entre les deux stations sont attribuables à l'existence de profondeurs différentes de brassage des eaux et à l'effet sur le régime d'éclairement de l'eau. Dans le bassin est, et avant la stratification thermique, la prolifération phytoplanctonique était limitée par la teneur en phosphore et en silice, alors que dans le milieu du lac, la lumière constituait le facteur limitant jusqu'au moment de la stratification. Les chercheurs ont estimé à 4–6 $mE \cdot m^{-2} \cdot min^{-1}$ le degré d'éclairement énergétique dans la colonne d'eau ($\bar{I}_{lim}$) à partir duquel la lumière constitue un facteur limitant la prolifération phytoplanctonique. Le $\bar{I}_{lim}$ a été observé à une profondeur de brassage vertical de 38–50 m à une extinction de la lumière moyenne saisonnière de 0,30 m^{-1} par une journée sans nuage, soit le 21 juin. Les ratios de limitation par la lumière, soit le rapport entre l'éclairement fourni ($\bar{I}$) et l'éclairement requis défini par I_k (degré d'éclairement énergétique correspondant à l'atteinte de l'intensité de saturation lumineuse) ont été rapprochés des tendances saisonnières de l'indice de carence en phosphate.
[Traduit par la Rédaction]

Introduction

Phosphorus (P) management was implemented in the Great Lakes basin in the late 1970s because of concern over eutrophication of lakes Ontario and Erie (International Joint Commission 1969). Deteriorating water quality and high biomass of the nuisance alga *Cladophora* spp. in nearshore regions by the late 1960s clearly demonstrated the need for a management program to reduce P loading to Lake Ontario. Compared with the nearshore, offshore chlorophyll levels were not high (Stadelmann and Fraser 1974) and water quality problems were not as acute. Consequently, the P control program in Lake Ontario has sparked an ongoing debate on the extent to which light rather than P supply controls offshore phytoplankton growth. A complete understanding of the seasonal transition between these growth-limiting factors can help put the relevance of the P control program for offshore phytoplankton growth into perspective.

Previous studies have described the importance of thermal stratification to the seasonal transition from light to P limitation of phytoplankton growth (Stadelmann et al. 1974; Lean et al. 1987). Unfortunately, these studies did not provide details on factors limiting water column irradiance such as mixing depth, light extinction, incident irradiance, and physiological demand for light. Our first goal was to seasonally quantify these factors to predict whether light should be limiting to phytoplankton growth under various environmental conditions. This predictive capability could be useful to researchers who need information on the P deficiency status of the plankton but lack direct measurements. Spatial differences in spring diatom development were examined as part of this goal to show how small differences in water column irradiance

Received January 1, 1995. Accepted December 7, 1995.
J12736

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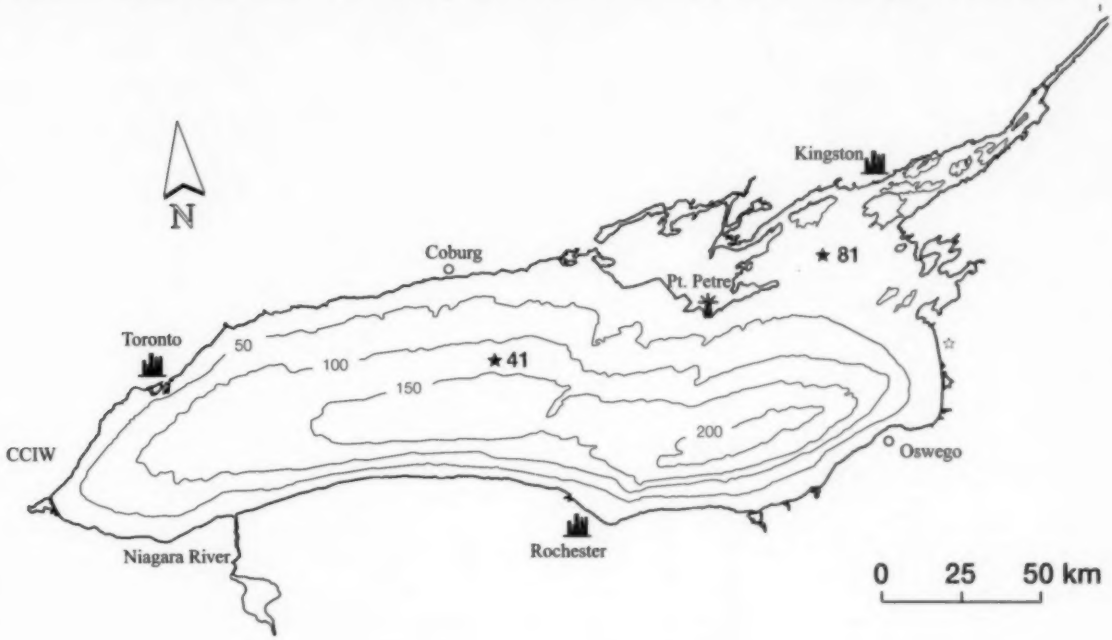
Fig. 1. Lake Ontario long-term biomonitoring stations: 81 in the eastern basin (depth = 34 m) and 41 in midlake (depth = 126 m). Solar radiation monitoring locations at Point Petre, Ontario, and the Canada Centre for Inland Waters (CCIW), Burlington, Ont., are shown.



can affect nutrient limitation of phytoplankton during critical parts of the seasonal cycle.

Our second goal was to determine whether seasonal trends in the P deficiency of offshore phytoplankton had changed since this variable was last measured in 1982 during the Lake Ontario Nutrient Assessment Study (LONAS) (Lean 1987; Lean et al. 1987). Had the phytoplankton become increasingly P deficient as offshore P levels declined through the 1980s in response to continued P control (Johengen et al. 1994)? If a primary goal of P management was to make this nutrient more limiting to phytoplankton growth, then P deficiency of these organisms should be periodically assessed. Similar views have been expressed by other researchers on the importance of monitoring light and P limitation of phytoplankton growth in Lake Ontario (Hartig et al. 1991). Our study expanded on the LONAS study spatially by sampling the midlake and the shallower but important (from a fisheries perspective) eastern basin and temporally by conducting the study for 2 consecutive years.

The study reported in this paper was part of the long-term Lake Ontario Biomonitoring Program (Bioindex Project, Department of Fisheries and Oceans, Burlington, Ont.). A companion paper reports on recent trends and historical changes in phytoplankton photosynthesis in Lake Ontario (Millard et al. 1996).

Methods

Field sampling

Locations in this study have been sampled extensively since 1981 (Johannsson et al. 1985). Two locations were sampled weekly for nutrients, temperature, light, phytoplankton and zooplankton biomass, and photosynthesis from late April to late October, but phytoplankton P deficiency was measured in alternate weeks (Fig. 1). Composite samples were collected with an integrating sampler from the surface to 20 m under isothermal conditions or weak thermal stratification and from the surface to 1 m less than the epilimnion depth during thermally stratified conditions. Samples were placed in acid-washed, darkened, insulated 20-L polycarbonate bottles. The bottles were well rinsed with sample water at collection time. Subsamples for measurement of nutrients, chlorophyll, biomass, P demand, and photosynthesis were taken from the same bottle.

Solar radiation, temperature, and light extinction

Temperature profiles were measured using an electronic bathythermograph and converted to density to determine vertical mixing depth (Z_m) (see Table 1 for definitions of abbreviations). The start of the metalimnion was defined as the first depth interval (0.5 m) where the density gradient exceeded 0.08 $kg \cdot m^{-3} \cdot m^{-1}$ (Harris et al. 1980; Reynolds and Wiseman 1982; Reynolds et al. 1984; Millard 1986*a*). High-density gradients at depths of less than 4 m were considered transient, and mixing depths this shallow were not chosen. In these instances, a deeper density gradient that exceeded the criteria for a metalimnion was selected if present, otherwise the water column was sampled as if isothermal.

In situ attenuation of photosynthetically available radiation (PAR) was measured using an LI–192 underwater, cosine-corrected quantum sensor and an LI–190 quantum deck cell (LI-COR, Inc., Lincoln, Neb.). Deck cell readings were used to normalize underwater values measured under variable cloud cover. The extinction coefficient (ε_{par}) was calculated as the slope of a linear regression of the natural logarithm of light intensity versus depth. Incident solar irradiance was recorded continuously at each end of the lake using LI–190 quantum sensors (LI-COR, Inc.) and dataloggers. Mean epilimnetic irradiance ($\bar{I}$) was calculated by the computer programs of Fee (1990) (see Millard et al. 1996 for further details on light measurements). $\bar{I}$ calculated with cloudless irradiance has been used to show the influence

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Table 1. Nomenclature.

	Definition	Units
PAR	Photosynthetically available radiation (400–700 nm)	
I_o	Incident solar radiation.	$mE \cdot m^{-2} \cdot min^{-1}$
ε_{par}	Vertical extinction coefficient for PAR	m^{-1}
Z_m	Vertical mixing depth	m
Z_{eu}	Depth of euphotic zone; 1% penetration of I_o	m
$\bar{I}$	Mean irradiance of PAR in the epilimnion	$mE \cdot m^{-2} \cdot min^{-1}$
$\bar{I}_{day}$	$\bar{I}$ calculated for the daylight period	$mE \cdot m^{-2} \cdot min^{-1}$
$\bar{I}_{24}$	$\bar{I}$ calculated for a 24-h period	$mE \cdot m^{-2} \cdot min^{-1}$
I_k	Irradiance of PAR defining the onset of light saturation of photosynthesis; photosynthesis below I_k is light limited	$mE \cdot m^{-2} \cdot min^{-1}$
P_{opt}	Photosynthetic rate at optimal PAR	$mg\ C \cdot m^{-3} \cdot h^{-1}$
V_{max}	Maximal uptake velocity of orthophosphate	$mg\ P \cdot m^{-3} \cdot h^{-1}$
PDI	Phosphorus deficiency index (P_{opt}/V_{max})	None
τ_{PO4}	Turnover time of orthophosphate	min
LLR_{24}	Light limiting ratio ($\bar{I}_{24}/I_k$)	None
LLR_{day}	Light limiting ratio ($\bar{I}_{day}/I_k$)	None
$\bar{I}_{lim}$	Limiting value of $\bar{I}$; P demand is low below this value, indicating light limitation of phytoplankton growth rates	$mE \cdot m^{-2} \cdot min^{-1}$

of Z_m and ε_{par} on the underwater light climate because it removes variability in incident solar irradiance (I_o) caused by clouds. $\bar{I}$ calculated with measured irradiance has been used to analyze the phytoplankton response to actual light conditions in the water column. Seasonal trends in $\bar{I}$ were calculated on a 24-h basis ($\bar{I}_{24}$), the default method of calculation by the Fee (1990) programs, for comparison with other studies (Hecky and Guildford 1984; Fee et al. 1992; Guildford et al. 1994). Calculations were also made for the daylight period only ($\bar{I}_{day}$).

Phosphate turnover time, P_{opt}, and V_{max}

Turnover time of orthophosphate (τ_{PO4}) is the time required by the plankton to take up an amount of P equivalent to that present. Rapid τ_{PO4} values of under 10 min indicate extreme P deficiency in the plankton whereas longer τ_{PO4} values of hours to days indicate no P deficiency (Lean and Nalewajko 1979; Lean and Pick 1981). τ_{PO4} was determined by adding prefiltered, carrier-free, $^{32}PO_4$ to 100 mL of unfiltered lake water in polycarbonate flasks to yield a final concentration of 0.33–1.67 $kBq \cdot mL^{-1}$ (20 000 –100 000 $dpm \cdot mL^{-1}$). Samples were incubated in an ambient-temperature water bath under low laboratory light (<30 $\mu E \cdot m^{-2} \cdot s^{-1}$; 1 $\mu E \cdot m^{-2} \cdot s^{-1} = 0.217\ W \cdot m^{-2}$). Five-millilitre aliquots were filtered through 0.2-μm Nuclepore polycarbonate filters at successive times following isotope addition. Sampling times were adjusted according to the anticipated τ_{PO4} such that intervals between filtrations were shortest when τ_{PO4} was most rapid. Samples were incubated longest in the spring and fall (1–3 h) when uptake was slowest. Filters were given a small rinse of about 0.5 mL of unlabelled lake water at the end of the filtration to remove residual water and unincorporated tracer. Filters were solubilized with 100–200 μL of a 10:1 mixture of methylene chloride and ethanolamine. Ten millilitres of Redi-Gel scintillation fluor (Beckman) was added and the samples were counted by liquid scintillation for either 10 min or to a 2 sigma accuracy of 2%. Quench was never a problem because of the high beta energy of ^{32}P.

The natural logarithm of the percentage of isotope left in the filtrate was regressed against time and the initial slope was used to approximate the rate constant (k) for P uptake. Values of r^2 were usually >0.95 with an intercept close to 100%, indicating that radiotracer was added in solution. The reciprocal of the rate constant is τ_{PO4}.

The P deficiency index (PDI) (Lean and Pick 1981), the ratio of the optimal rate of carbon uptake at light saturation (P_{opt}) divided by the maximal uptake velocity of P (V_{max}), was used to assess whether the phytoplankton were P limited. Complete details of phytoplankton photosynthesis in Lake Ontario are given in the companion paper (Millard et al. 1996). PDI values were scaled according to the scheme of Lean and Pick (1981) where values >100 indicate either no or a low level of P deficiency. Values in this range are indicative of carbon (C) and P uptake proportional to intracellular ratios of these elements required for phytoplankton growth in oligotrophic waters (Harris 1986). Values of 30–100 indicate low, values of 10–30 moderate, and values <10 extreme, P deficiency and high demand for P. P-depleted plankton respond to additions of P with preferential uptake of P compared with C, causing the PDI to decline. Suppression of C uptake in P-starved plankton (Lean and Pick 1981; Lean et al. 1987) was not an issue in our experiments because V_{max} was estimated in separate bottles from those used for photosynthesis. Phytoplankton P demand increases as cellular stores of P decline and the phytoplankton become P deficient relative to their growth requirements (Rhee 1973; Healey and Hendzel 1975; Healey 1979; Lean and Pick 1981). P demand measurements can indicate whether phytoplankton growth is P limited.

V_{max} is the uptake velocity ($k \times$ P concentration) measured at saturating levels of P and is reached when rates do not increase with further additions of P. In 1988, V_{max} was estimated from a linear transformation of the Michaelis–Menten equation for enzyme kinetics relating uptake of a limiting nutrient to added substrate concentrations. The equation used to relate τ_{PO4} to added P was

$$(1) \qquad \tau_{PO4} = \frac{(K_t + P_n)}{V_{max}} + \frac{P_a}{V_{max}}$$

where K_t is the half-saturation constant, the P concentration at which uptake velocity is equal to $0.5V_{max}$, P_n is the ambient phosphate concentration; V_{max} is the maximum uptake velocity of P, and P_a is the added P concentration. P_n was not added to P_a when uptake was calculated for the enriched treatments because ambient orthophosphate levels are typically very low (Johannsson et al. 1985), are not measured accurately (Rigler 1966, 1968), and would have been negligible compared with enriched levels. In 1988, two to five concentrations ranging from 1.5 to 20 $\mu g \cdot L^{-1}$ were used to estimate V_{max}. The estimate of k at ambient P levels was used in the plot along with a low P_n (0.1 $\mu g \cdot L^{-1}$) to approximate the low uptake at average ambient conditions. Earlier studies in Lake Ontario showed that V_{max} was usually reached at about 10 $\mu g \cdot L^{-1}$ (Lean and White 1983), a conclusion supported by our study. As a result, a simpler method of

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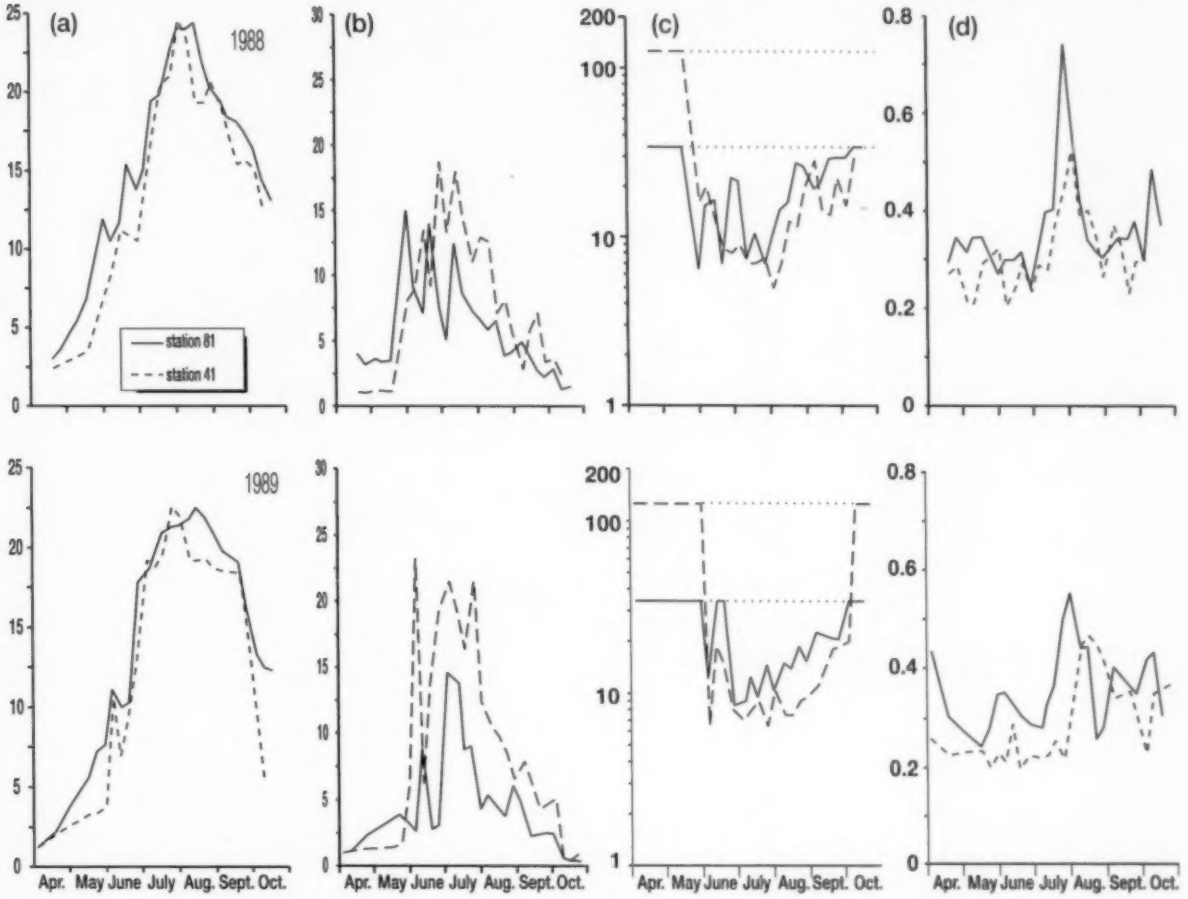
Fig. 2. Seasonal trends in (*a*) temperature, (*b*) $\bar{I}_{24}$ (mE·m^{-2}·min^{-1}), and its determinant factors (*c*) Z_m (m) and (*d*) ε_{par} (m^{-1}). The horizontal broken lines in *c* denote the station depth for stations 41 (126 m) and 81 (34 m). $\bar{I}$ was calculated with theoretical cloudless irradiance on a 24-h basis.



determining V_{max} was used in 1989, where k was estimated at 20 and 30 μg·L^{-1}, concentrations that saturated P uptake in 1988. V_{max} was estimated directly from these experiments as k times the elevated P concentration. The average of the two estimates was usually used because both estimated V_{max}. These simpler estimates of V_{max} in 1989 showed excellent agreement with V_{max} estimated with eq. 1 ($Y = 0.98X + 0.037$, $r^2 = 0.997$, $n = 27$), indicating that estimates of V_{max} in 1989 were as accurate as those in 1988.

Silica and diatom biomass

Silica analyses were conducted on filtered (0.45 μm) water samples by the National Water Quality Laboratory, Environment Canada, Burlington, Ont., using standard methodology (Environment Canada 1979). Whole-water samples from the integrator were preserved with Lugol's solution and phytoplankton were enumerated and measured using the Utermohl inverted microscope technique for estimates of diatom biomass (Utermohl 1958; Johannsson et al. 1985).

Results

Mean epilimnetic irradiance and temperature

$\bar{I}$ was lowest in the spring and fall at both stations when the water column was fully mixed to the bottom (Fig. 2). $\bar{I}$ was distinctly higher at station 81 during April and May at full mixing (1–3 mE·m^{-2}·min^{-1}) compared with station 41 (<1 mE·m^{-2}·min^{-1}) because of the shallower depth. $\bar{I}$ increased following thermal stratification at both stations to >10 mE·m^{-2}·min^{-1} as Z_m shortened and ε_{par} remained low. $\bar{I}$ declined in late July or early August at both stations owing to the combined effects of declining solar irradiance, the annual summer increase in ε_{par} resulting from whiting (Millard et al. 1996), and a gradual deepening of Z_m to the permanent stratified depth of 20 m (Boyce et al. 1989). Fall values of $\bar{I}$ at full vertical mixing at station 81 were lower than in the spring because ε_{par} was higher. Lower incident irradiance in the fall was a factor for $\bar{I}$ calculated with empirical irradiance. Annual and spatial differences in fall $\bar{I}$ are due to differences in breakdown of stratification and deepening of Z_m.

Generally, seasonal trends in mean epilimnetic temperature were similar between stations, but station 81 warmed more rapidly and reached higher temperatures in the spring than station 41 because of the shallower depth (Fig. 2). Mean

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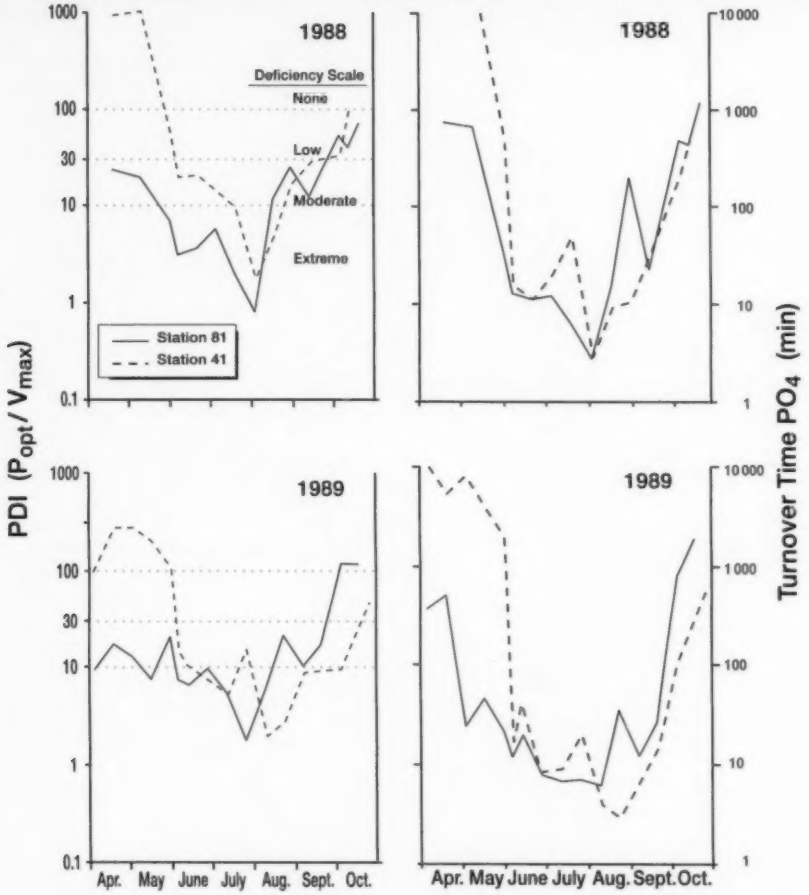
Fig. 3. Seasonal trends in the phosphate deficiency index (PDI), the ratio of P_{opt}/V_{max}, and the turnover time of orthophosphate.



summer epilimnetic temperatures were higher at both stations in 1988 than in 1989.

Phosphorus deficiency index

High values of the PDI at low I indicate that light not P is the primary growth-limiting factor. High PDI at higher light levels should occur only in eutrophic lakes where P supply can exceed growth requirements. The PDI was >100 at station 41 in the spring, the result of very low V_{max} (<0.1 mg $P \cdot m^{-3} \cdot h^{-1}$), an indication that phytoplankton growth rates were also low (Fig. 3). In contrast to midlake, the PDI at station 81 indicated extreme to moderate P deficiency in the early spring, although the water column was mixed to the bottom (34 m). Spring V_{max} at station 81 was much higher than at station 41, but rates were still low compared with the summer values.

The PDI declined markedly with the onset of thermal stratification at both stations as V_{max} increased relative to P_{opt}. The summer minima in the PDI at both stations were the result of the extreme V_{max} values (>20 mg $P \cdot m^{-3} \cdot h^{-1}$) in late July and early August of both years. The extreme summer V_{max} values are among the highest values found in the literature (Lean and White 1983; White and Payne 1980) and are indicative of extreme P deficiency. Variability in V_{max} rather than P_{opt} was the primary factor for variability in the PDI, similar to previous studies (Lean et al. 1987). P_{opt} also increased in the summer with higher rates occurring at station 81 (15–25 mg $C \cdot m^{-3} \cdot h^{-1}$) than at station 41 (12–20 mg $C \cdot m^{-3} \cdot h^{-1}$) (Millard et al. 1996). In spite of the increase in P_{opt} during the summer, the PDI still decreased because of the comparatively greater increase in V_{max}. The PDI remained below 10 throughout the summer at both locations but increased in late August as Z_m deepened and the shift to light-limited growth started. The late summer increase in the PDI started earlier at station 81 in both years compared with midlake because thermal stratification eroded earlier. At station 81 the PDI indicated less deficiency at full mixing in early October compared with the same mixing conditions in the spring.

Orthophosphate turnover time

Both τ_{PO4} (Prepas 1983; Planas and Hecky 1984; Millard 1986*b*) and PDI (Lean et al. 1983, 1987; Istanovics et al. 1992) have been used in other studies as indices of P demand. Trends in both indices have been shown because each provides different information about the P status of the plankton. The PDI is

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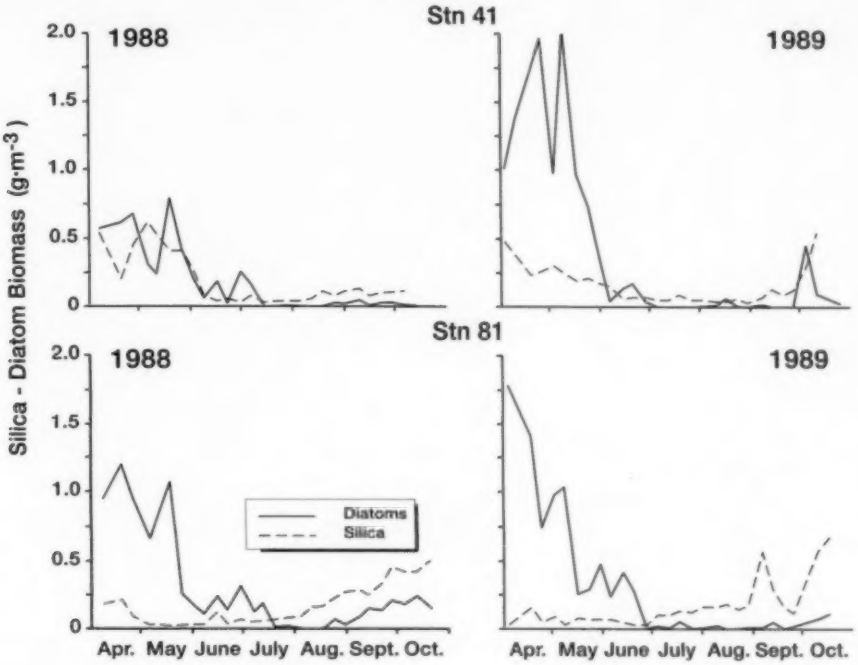
Fig. 4. Seasonal trends in silica concentration and diatom biomass at station 41 in the midlake and station 81 in the eastern basin of Lake Ontario.



independent of biomass and reflects the deficiency of the phytoplankton because uptake of bacteria is saturated by artificially elevating levels of P. On the other hand, τ_{PO4} is determined at ambient levels of P, reflects the in situ P status of the entire plankton, and is influenced by levels of P and biomass (Lean and Nalewajko 1979), P content per cell (Gotham and Rhee 1982), and the size of the plankton (Lean 1984; E.S. Millard, unpublished data). The relationship between the PDI and τ_{PO4} in our study ($\log_{10}$ PDI = 0.539($\log_{10}$ τ_{PO4}) + 1.088, r = 0.902, n = 59) was almost identical to that found by other researchers (Lean et al. 1987; Istanovics and Herodek 1985; Istanovics et al. 1992). Agreement between these two indices of P demand was not as close during the spring in the eastern basin of both years and in midlake in the fall of 1988.

The seasonal pattern in τ_{PO4} at both stations was similar to that previously observed in Lake Ontario (Lean et al. 1987) (Fig. 3). τ_{PO4} ranged from 7 days in the early spring to only 2–3 min by midsummer. Although τ_{PO4} was faster at station 81 than at station 41 in the spring, τ_{PO4} at station 81 was as slow as several hours, indicating low P demand. τ_{PO4} in excess of 100 min would usually indicate low demand for P (Lean and Pick 1981; Millard 1986*b*). In contrast to trends in τ_{PO4}, the PDI at station 81 indicated extreme P deficiency as early as April in 1989. The PDI is a better indicator of P deficiency in these situations and use of only τ_{PO4} as an index of P demand would have led us to conclude that the phytoplankton were not P deficient at this time.

Similar to the PDI, τ_{PO4} decreased rapidly (<10 min) following thermal stratification during the summer but increased by late summer and into the fall as Z_m deepened. The difference between spring and fall τ_{PO4} at station 81 was less pronounced than with the PDI.

Silica and diatom biomass

There were important differences between the two stations in the pattern of spring diatom development and its effect on pools of available silica and P. Diatom biomass was highest and most variable at station 41 in April and May but declined abruptly with thermal stratification in early June (Fig. 4). At station 81 diatom biomass was already in decline in early April when sampling started and continued to decline through May prior to thermal stratification. The trends in spring diatom biomass at both stations were parallelled by similar trends in chlorophyll concentration (Millard et al. 1996). Diatom biomass was negligible during the rest of the season at both stations except for a slight increase in the fall.

Spring diatom populations are known to deplete silica to growth-limiting levels (Lund 1954, 1955). The difference in spring silica concentrations between the two regions of the lake reflects the difference in growth activity between the two populations, a consequence of the more favourable light environment in the shallower eastern basin. Spring silica levels were considerably higher at station 41 than at station 81, where concentrations were less than 0.100 mg·m^{-3} by the end of April. Higher diatom biomass at station 41 in 1989 caused silica levels to be in constant decline with lower mean concentrations throughout the spring (0.275 ± 0.100 mg·m^{-3}) compared with 1988 (0.453 ± 0.130 mg·m^{-3}). Silica levels at both stations remained very low throughout both summers (<0.100 mg·m^{-3}) with slight increases in the fall.

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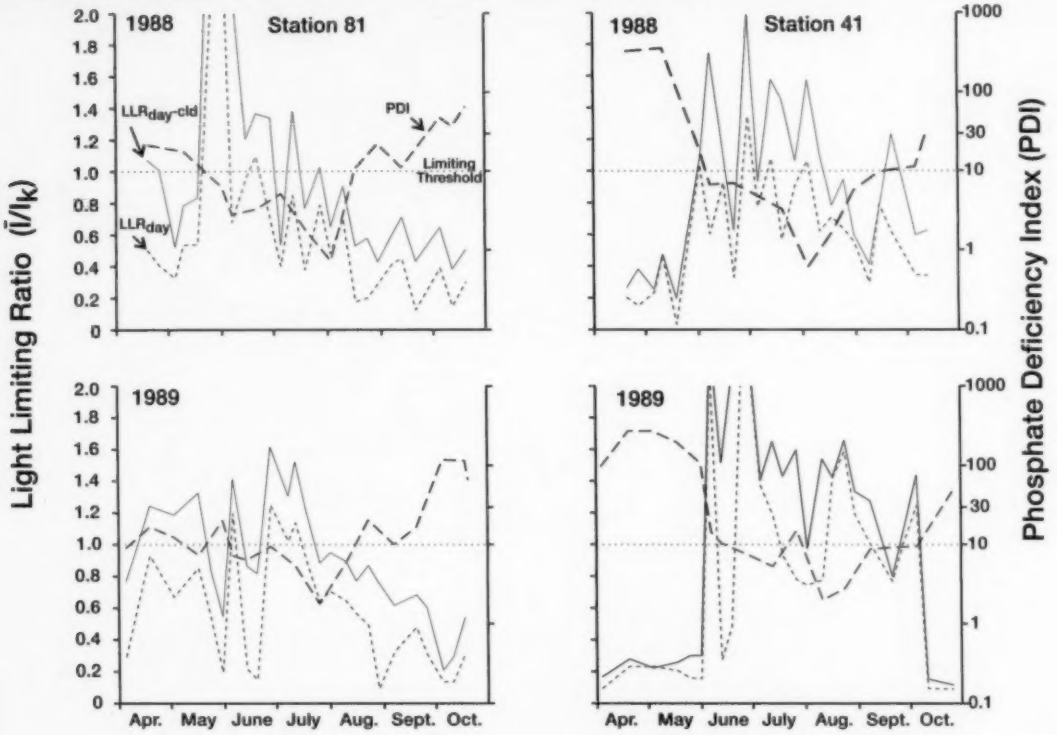
Fig 5. Seasonal trends in light-limiting ratios (LLR = $\bar{I}/I_k$). LLR_{day} was determined using I calculated on a daylight basis with empirical solar irradiance. LLR_{day}-cld was determined with I calculated on a daylight basis using theoretical cloudless irradiance.



Light supply and demand: light-limiting ratio

Light limitation of phytoplankton growth was assessed with a supply (I) / demand (I_k) ratio for PAR, hereafter referred to as the light-limiting ratio (LLR). Light demand of the phytoplankton was characterized with I_k, the light saturation parameter for photosynthesis. Irradiance levels above I_k were saturating for photosynthesis, and values below I_k were limiting. The supply aspect of the LLRs was calculated with various I values to determine which ratio agreed best with trends in the PDI (Fig. 5). Theoretically, when the ratio is >1, growth is not light limited because supply exceeds demand and the PDI will decline to the extreme range (<10) because P supply is low during the thermally stratified period. Rapid summer τ_{PO4} indicates that Lake Ontario is a low P supply environment. Conversely, the PDI should rise to >30 when the LLR is <1, indicating that light is the predominant growth-limiting factor.

LLR was determined using I calculated with empirical solar irradiance on both a 24-h (LLR_{24}, not shown in Fig. 5) and daylight basis (LLR_{day}), and also with cloudless irradiance (LLR_{day}-cld) to show maximal values for the LLR. The influence of cloudy weather on the sampling day was evident on several occasions where there was a large discrepancy between LLR_{day} and LLR_{day}-cld (e.g., mid-June 1989). Fluctuations in the LLRs can be caused by changes in factors affecting the supply (I = interaction of I_o, Z_m, ε_{par}) or demand ($I_k = P^B_m/\alpha^B$) sides of the light ratio. Millard et al. (1996) provide a thorough description of the seasonal interaction between the photosynthetic parameters P^B_m and α^B and their influence on I_k in Lake Ontario.

Station 41

The LLR_{24} was calculated but not shown on the graph because trends in the PDI showed better agreement with the LLR_{day}. The LLR_{24} was rarely close to or above 1 at station 41 during the study although the PDI indicated the phytoplankton were P, not light, deficient and the ratio should have been higher (e.g., mid-July to August in 1989). In contrast, LLR_{day} exceeded or was close to 1 from early June to early August in 1988 when the PDI was low. Over the whole season, changes in the LLRs were driven primarily by changes in Z_m that affected the supply side of the ratio. However, the consistent increase in the demand side of the LLR during the summer of 1989 as I_k rose to over 300 $\mu E \cdot m^{-2} \cdot s^{-1}$ combined with I_o well below cloudless levels on sampling dates in July and August were important factors in the summer decline in the LLR_{day} (Millard et al. 1996).

The late summer and fall PDI values fluctuated because of the effect of variable mixing depths on I. Deeper mixing lowered the LLR during this period in 1988, and the effect was exacerbated by higher average I_k (239 ± 25 $\mu E \cdot m^{-2} \cdot s^{-1}$) compared with 1989 values (169 ± 27 $\mu E \cdot m^{-2} \cdot s^{-1}$). The LLR_{day} dropped well below 1 in October in both years at station 41 as stratification broke down and Z_m extended to the bottom and the PDI rose as expected.

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Fig. 6. Relationship between the phosphate deficiency index (PDI) and mean epilimnetic irradiance ($\bar{I}_{day}$) calculated using empirical solar irradiance on a daylight basis. $\bar{I}_{lim}$ is an estimate of the critical value of $\bar{I}_{day}$ determining the transition between light and phosphorus limitation of phytoplankton growth. The horizontal broken line at $\bar{I}_{day} > \bar{I}_{lim}$ is the mean of all corresponding PDI values. At $\bar{I}$ below $\bar{I}_{lim}$ the broken line is a fitted log-linear regression ($\log_{10}$PDI = −0.243 $\bar{I}_{day}$ + 2.175, n = 30, r = 0.54). Asterisks represent dates where PDI was atypically in the extreme range for $\bar{I}_{day}$ below $\bar{I}_{lim}$. See text for further details.

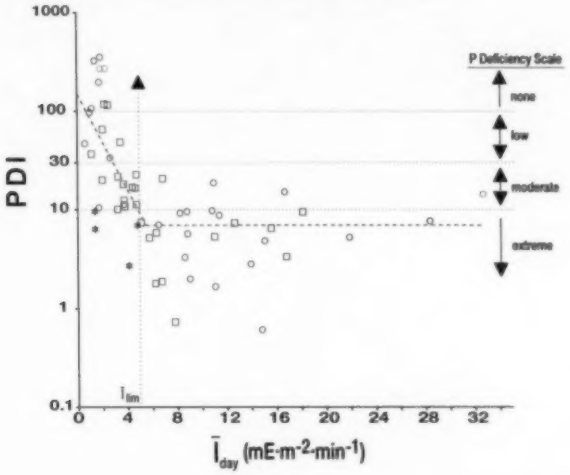
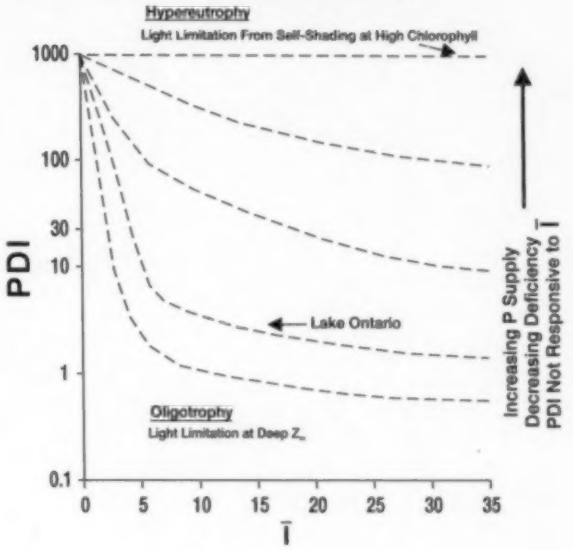


Fig. 7. A conceptual model of the relationship between the phosphorus deficiency index (PDI) and mean epilimnetic irradiance ($\bar{I}$) at varying levels of phosphorus supply.



Station 81

The relationship between the LLR_{day} and the PDI at station 81 was not as consistent as that at the midlake. For example, during April and May of 1988 the LLR_{day} was below 1 yet PDI showed moderate P demand. Only the LLR_{day}-cld consistently showed the expected agreement with seasonal trends in the PDI. The large fluctuations in the LLRs in June 1989 were due to inconsistent stratification patterns and changes in Z_m. Similar to station 41, on some occasions light demand influenced LLR_{day}. In April and May of 1989, LLR_{day} increased over 1988 values for the same period owing to a 25% decrease in I_k and not to a change in light supply. Similar to the findings for the midlake, the PDI steadily increased during the late summer and fall as LLR_{day} declined to <1, indicating a return to light-limited growth as Z_m deepened.

A critical value for $\bar{I}$

A critical value for $\bar{I}_{day}$, hereafter referred to as $\bar{I}_{lim}$, below which growth was considered to be light limited was estimated from a plot of PDI versus $\bar{I}_{day}$ (Fig. 6). $\bar{I}_{lim}$ was difficult to establish using conventional curve-fitting techniques. At values of $\bar{I}_{day}$ above the range of 4–6 mE·m^{-2}·min^{-1}, PDI did not respond to light and most values fell in the extreme range. We considered 5.0 mE·m^{-2}·min^{-1} to be a reasonable approximation for $\bar{I}_{lim}$on the basis of the distribution of points. Ideally, if our estimate of $\bar{I}_{lim}$ was accurate, at $\bar{I}_{day}$ below $\bar{I}_{lim}$, PDI should have been >10 and at $\bar{I}_{day}$ above $\bar{I}_{lim}$, <10.

Below our estimate of $\bar{I}_{lim}$ the PDI responded strongly to light and all but four points had PDI > 10. These atypical points were included in the regression, although their distribution warrants explanation. Three of the points were misplaced on the plot because the levels of irradiance eliciting the high P demand were higher than those measured at the time the PDI was measured. For example, two PDI estimates were made when there were very cloudy conditions ($\bar{I}_{day}$ only 30% of cloudless values) on sampling days in early June shortly after the onset of thermal stratification. On these days the PDI was likely responding to a recent light history of higher $\bar{I}$ caused by the abrupt change in Z_m at stratification and not to the lower $\bar{I}$ on the sampling day. On another occasion at station 81 (June 1989), $\bar{I}$ was probably actually higher than the value used on the plot because mixing depth was overestimated. This occurred because thermal stratification was not well established during June 1989, and as a result, Z_m was difficult to determine. The fourth atypical PDI estimate occurred at station 81 and had a value for $\bar{I}_{day}$ (3.98 mE·m^{-2}·min^{-1}) just below $\bar{I}_{lim}$, suggesting that from a seasonal perspective a range should be considered for $\bar{I}_{lim}$ (4–6 mE·m^{-2}·min^{-1}).

Above our range for $\bar{I}_{lim}$ all but four PDI values were in the extreme range of P deficiency, and these values were in the lower part of the moderate demand range (10–20). The average PDI above $\bar{I}_{lim}$ was well down in the extreme category of P deficiency (6.94 ± 5.1, n = 29, range = 0.61–20.5).

Discussion

Light-limiting ratios and the PDI versus light relationship

There is a complex relationship between the PDI, light, and P supply depicted as a conceptual model in Fig. 7. The relationship from Lake Ontario is only one of a potential family of PDI response curves to light supply. At the other trophic extreme, high P supply will satisfy growth demand at all light levels and the PDI does not respond to changes in $\bar{I}$. It is not clear what

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happens to I_{lim} at increasing levels of both P and light at this point in our understanding. Healey (1985) found in culture work that nutrients and light could simultaneously limit phytoplankton growth and that the increased supply of one factor could compensate for a decrease in the other. However, his study was carried out over a narrow range of suboptimal irradiances compared with the wide range depicted in the model. The model indicates that I_{lim} increases and becomes less distinct at higher P supply. However, further studies measuring the PDI in eutrophic lakes are required before the relationship between P deficiency and light at higher levels of P supply is fully understood and the exact shape of other PDI response curves is known.

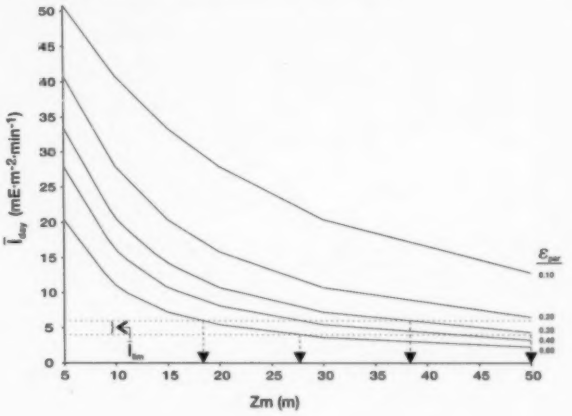
Defining limits to Z_m and ϵ_{par} for I_{lim}

The limiting Z_m for any I_{lim} and ε_{par} can be determined by interpolation on the nomogram that shows the relationships between the determinant variables for I_{day} (Fig. 8). The nomogram illustrates that for the range for I_{lim} of 4–6 mE·m^{-2}·min^{-1} at the seasonal mean ε_{par} for Lake Ontario of about 0.3 m^{-1} (Z_{eu} = 15.4 m) (Millard et al. 1996), Z_m could extend as deep as 38–50 m before light would limit phytoplankton growth. However, seasonal changes in irradiance or cloudy conditions will reduce the limiting Z_m compared with the nomogram, which calculated I with cloudless irradiance at the seasonal maximum (June 21). I_{lim} occurs at shallower mixing depths under cloudy conditions because I responds linearly to change in I_o. A decline in I_o of 50% is equivalent to doubling ε_{par} and I_{lim} would be reached at a Z_m of about 18–27 m (intersection of I_{lim} with ε_{par} = 0.60 curve). The duration of cloudy weather in the spring must be an important factor for diatom growth at station 81 during full spring mixing. At the lower range for I_{lim} (4 mE·m^{-2}·min^{-1}) and ε_{par} of 0.3 m^{-1}, I_o would have to be less than 50% of cloudless values before mixing to the bottom was critical at station 81. Whiting events will cause I_{lim} to be reached at comparatively shallow Z_m even under cloudless conditions because ε_{par} peaks at 0.4–0.6 m^{-1} (Millard et al. 1996).

Light-limiting ratios

The light supply in the mixed layer of lakes has been described previously by I (Riley 1957; Sterner 1990). However, researchers have been inconsistent on whether to calculate I for a 24-h (Hecky and Guildford 1984; Fee et al. 1992; Guildford et al. 1994) or daylight period (Riley 1957; Oliver and Ganf 1988; Neale et al. 1991; Carignan and Planas 1994). I calculated on a 24-h basis removes the seasonal and latitudinal influence of day length on I. Most of the change in I_{day} and the LLRs was due to changes in Z_m and I_o and secondarily to changes in I_k and ε_{par}. However, I_k did significantly influence LLRs during a few selected periods. In our study, LLR_{day} was the better descriptor of the two LLRs using empirical irradiance, particularly at station 41. The LLR_{24} did not accurately predict light limitation and was frequently <1 in our study, suggesting that phytoplankton were light limited when the PDI was <30. Fee et al. (1992) found that LLR_{24} was rarely above 1 in large lakes (>100 ha) in their lake-size series (Fee and Hecky 1992) and concluded that phytoplankton growth was often light limited, although measures of P demand were not available at the time to support this conclusion. More recently Guildford et al. (1994), studying the same lake-size series,

Fig. 8. A nomogram relating mean epilimnetic irradiance (I) to mixing depth (Z_m) at varying degrees of transparency (ε_{par}). Limiting values of Z_m are shown at the intersection of I_{lim} with the light extinction curves. Calculations were made on a daylight basis using theoretical cloudless irradiance on June 21.



showed that phytoplankton were P limited even in the larger lakes but not as severely as in the smaller lakes. This suggests that I_{day} may be a better descriptor of the light levels required to overcome limitation in their lakes than I_{24}. LLRs may be useful to ecologists who need to predict light limitation but lack sophisticated measures of nutrient demand (Fahnenstiel and Scavia 1987). However, the critical value of an LLR determining the transition from light to P limitation may be unique to each lake. In theory, Lake Ontario phytoplankton should have exhibited extreme P deficiency (PDI < 10) only when the LLR was >1; however, critical values may vary seasonally because of changes in species composition and complex interactions with nutritional status of the phytoplankton community at low light levels (Healey 1985). Theoretically, the critical LLR is 1 but the effective value for LLR_{day} appears to be closer to 0.8 for Lake Ontario during the stratified period. Late summer and fall trends in the PDI showed that once the LLR_{day} fell below 0.7, the PDI increased because light had definitely become the primary growth-limiting factor.

Variability in the effective LLR in Lake Ontario may have been due to errors in estimating Z_m in the early spring and fall when stratification is weak. In the spring, the effective Z_m can be significantly less than that indicated by temperature profiles. An example from June 6, 1988, at station 41 will illustrate this point. We concluded that on the basis of our density criteria, a thermocline was not present, although some warming had occurred in the top 20 m, causing the weak temperature gradient. However, if mixing had been to the bottom an isothermal profile should have been present. I was calculated using station depth as the mixing depth when a thermocline was not present. τ_{PO4} measured on a surface to 14-m composite sample indicated that this would have been a misleading approach. τ_{PO4} for the composite sample was only 15.6 min, suggesting that the phytoplankton were responding to light in a mixed layer much shallower than the station depth. The previous week, τ_{PO4} was 498 min for a surface to 20-m sample and the isothermal temperature profile indicated that mixing

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was to the bottom. τ_{PO4} could not have declined so drastically if the water column had remained fully mixed. The change in mixing regime must have been short term because surface temperatures were similar on both dates. If we had assumed that vertical mixing extended to the bottom on the second date, I would have been biased to a much lower value than that causing the rapid τ_{PO4}. Transient calm weather in the late spring and early summer can induce a shallower but temporary Z_m and higher effective I than that indicated by temperature profiles. Application of criteria for selecting mixed depth must be applied with caution because estimated and biologically effective mixing depths may differ (Horn and Paul 1984).

Our analysis related PDI to LLRs that were calculated with solar irradiance on the sampling day. As phytoplankton track short-term environmental fluctuations over time scales of several days to a week (Harris 1980; Jones 1978) it is conceivable that measured PDIs were responding to a light history preceding the sampling day. For example, irradiance measured on a cloudy sampling day following a brief period of high irradiance would have resulted in LLRs that were lower than those eliciting a growth response. To test whether the PDI showed better agreement with LLRs reflecting recent light history of the phytoplankton we calculated a mean I for a 4-day period prior to and including the sampling day (not shown in Fig. 5). The influence of variable incident irradiance close to sampling days was smoothed with this procedure, although the LLR_{24} was still consistently low when the PDI indicated that values should have been higher.

We contend that the PDI versus I relationship is one of cause–effect in Lake Ontario. Predicting the effect of the underwater light climate on the PDI using LLRs is not as straightforward as the PDI versus I relationship. The ratios were affected by seasonal changes in several influential factors for phytoplankton growth whereas the PDI versus I relationship has a single independent variable. Values of I_{day} above I_{lim} (5.0 mE·m^{-2}·min^{-1}) all but eliminated light limitation and consequently caused the PDI to decrease to the extreme range of P deficiency. Hecky and Guildford (1984) also found that I above a limiting level was a requisite condition for P limitation but was not in itself able to cause the onset of P deficiency because of an interaction with P supply. They also concluded that P limitation should occur if the condition $I > I_k$ was met and the rate of P supply was not sufficient to meet demand. They estimated I_{lim} at 5–7 mE·m^{-2}·min^{-1}, very close to the estimate of I_{lim} for Lake Ontario. However, these authors went on to adjust their estimate of I_{lim} calculated on a daylight basis downward to a 24-h value (3.75–5.25 mE·m^{-2}·min^{-1}) to agree with findings in culture studies. Our range for I_{lim} appears to be lower than values determined for tropical environments (Carignan and Planas 1994).

Seasonal transition between light and P limitation

The seasonal patterns of thermal stratification and water clarity in Lake Ontario that determine the transition periods (spring and fall) between light and P limitation of phytoplankton growth have been summarized in Fig. 9. The transition periods coincide with the major seasonal changes in mixing regime.

Spring diatom growth in the shallower eastern basin was uncoupled from the deeper offshore regions of Lake Ontario owing to the difference in underwater light climate. Even at full mixing in the spring, I was high enough in the eastern basin to alleviate light limitation, although I values were near the lower end of the range for I_{lim}. I_{lim} for the spring diatom communities appears to have been lower than for phytoplankton communities at other times of the season. Higher spring water temperatures in the eastern basin may have contributed to higher diatom growth rates and the earlier onset of silica and P deficiency than in the midlake.

The dominance of diatoms in the spring phytoplankton in the eastern basin likely caused the discrepancy in the P deficiency status indicated by the PDI and τ_{PO4}. Diatoms are likely to have high V_{max} (Smith and Kalff 1982) and storage capacity at saturating levels of P, but they are not as capable of rapid τ_{PO4} because of their lower surface area : volume ratios. The paucity of small particles such as bacteria and picoplankton that dominate uptake when τ_{PO4} is rapid and the plankton is P deficient (Rigler 1956; Currie and Kalff 1984; Istanovics et al. 1992) made rapid τ_{PO4} unlikely.

There was a continual decline in diatom biomass at station 81 prior to thermal stratification that was likely due to silica and P limitation of growth rates. Diatoms can flourish at lower light and temperature conditions than other phytoplankton groups (Harris 1978). Silica uptake by diatoms can be significant under low light intensities, making it possible for diatom growth from late winter to early spring to deplete supplies of available P and silica (Schelske et al. 1986). These authors suggest that historical P enrichment is responsible for significant winter silica demand with severe early spring depletion similar to our observations. Spring silica levels prior to stratification were low enough (<0.4 mg·L^{-1}) to limit diatom growth at station 81 (Lund 1964; Parker et al. 1977; Johengen et al. 1994).

In contrast to the eastern basin, extremely low I values owing to deep mixing were limiting diatom growth in the midlake prior to thermal stratification. Consequently, silica demand was lower and concentrations higher than in the eastern basin prior to stratification, although concentrations were still close to or below growth-limiting levels (Scavia and Fahnenstiel 1987; Johengen et al. 1994). However, rates of biomass loss owing to grazing and sedimentation were also very low at this time of the year. As a result, diatom biomass accumulated at more constant levels prior to stratification than in the eastern basin.

Diatom biomass in the midlake did not decline rapidly until the onset of thermal stratification, a pattern consistent with observations in lakes Michigan, Erie, and Ontario (Scavia 1979; Scavia and Fahnenstiel 1987; Munawar and Munawar 1976, 1982). The decline in diatom biomass occurs because nutrient-limited growth rates cannot keep pace with sedimentation rates that increase with nutrient limitation and the reduced mixing (Tilman and Kilham 1976). The rapid decline in both the PDI and τ_{PO4} coincident with stratification indicates that the diatom community became extremely P deficient.

The increase in the PDI as Z_m deepened in August at station 81 indicated that either P supply increased as hypolimnion water was entrained into the epilimnion or light-limiting conditions had returned. Lean et al. (1987) observed an increase in fall PDI values in Lake Ontario, but these authors did not describe the light and nutrient conditions present at the time. They were inconclusive as to whether the increase in the PDI was due to changes in P supply or light climate. We suggest that P deficiency declined in the fall at station 81 because of

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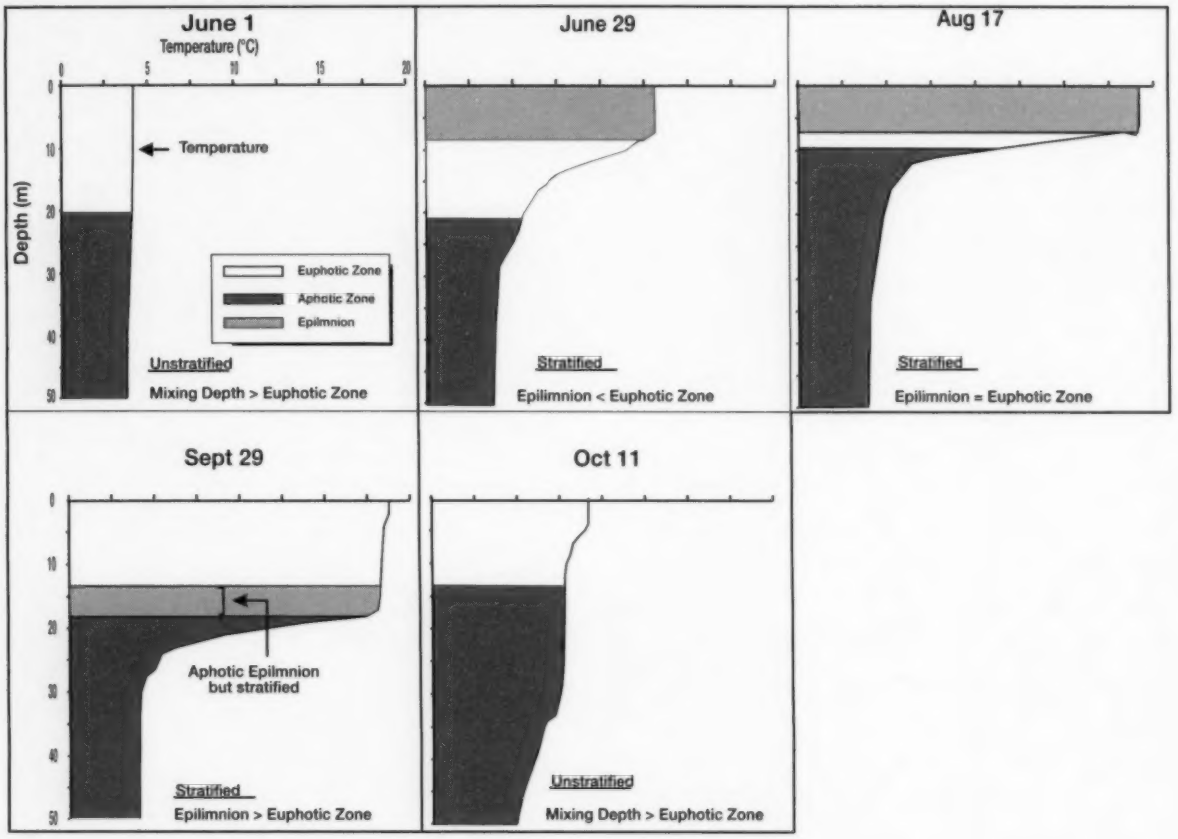
Fig. 9. The seasonal interaction between temperature, light, and mixing regimes for selected dates at station 41, 1989. On September 29 a portion of the epilimnion was below the euphotic zone under thermally stratified conditions. On October 11 the water column was not isothermal but density gradients were not sufficiently pronounced to delineate a thermocline.



light limitation of growth rates. A significant increase in P supply coincident with the breakdown in stratification was unlikely in our study because levels of soluble reactive P are not high in either the meta- or hypo-limnion at this time of the season (O. Johannsson, Bioindex Project, Burlington, Ont., unpublished data).

Relevance and future outlook

Our study describes in detail the environmental conditions that determine whether phytoplankton growth in Lake Ontario is light limited. Although light limitation extends over much of the year, seasonal PDI trends show that P management should control offshore phytoplankton levels during the extremely P-deficient summer period. P control is even more important to the eastern basin, where nutrient limitation of phytoplankton extends over a much longer period.

Seasonal trends in the PDI did not change in response to an additional 6 or 7 years of P control following the LONAS study, a pattern that reflects the relatively stable P loadings observed throughout the 1980s (Neilsen et al. 1994). Unless the human population in the drainage basin increases or the level of P control is reduced, these trends should remain similar in the future. Interannual variability of phytoplankton P deficiency is likely now controlled primarily by yearly variability in climatic factors rather than P loading, as suggested for phytoplankton photosynthesis (Millard et al. 1996). Future global climate change has the potential to alter seasonal transition between light and P limitation of phytoplankton growth by changing the timing and duration of thermal stratification, as well as epilimnetic depth and temperature (McCormick 1990). Our results show that P control would become increasingly important for controlling offshore productivity if the period of higher epilimnetic irradiance were longer.

Acknowledgments

We thank the captain and crew of the CSS *Bayfield* for safely guiding us through many days of rough weather and meeting our rigorous scheduling demands. Dave Lean and Ellen Bentzen provided technical advice at various stages of the project. Reviews of an earlier draft were provided by Stephanie Guildford, Dave Lean, and Ken Minns. Two anonymous reviewers provided very helpful comments that improved the manuscript. The advice and support of the staff of the Technical Operations section, Environment Canada, is gratefully acknowledged.

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Effects of planktivorous fish and nutrient additions on primary production of shallow versus deep (stratified) lake enclosures[1]

S. Perin, F.R. Pick, D.R.S. Lean, and A. Mazumder

Abstract: The effects of nutrients (N and P) and planktivorous fish on maximal photosynthetic rates and the size distribution of epilimnetic primary productivity were measured in both shallow (3.5 m) and deep (11 m) enclosures in an oligotrophic lake. The deep enclosures stratified in summer with an epilimnion and photic zone of the same depth as in the shallow, mixed, enclosures. The overall effect of nutrients and fish was the same in both shallow and deep enclosures. Nutrient enrichment caused phytoplankton productivity to increase about 10-fold both in the presence and absence of fish, but the presence of fish alone resulted in a 2-fold increase in total productivity in both nonfertilized and fertilized enclosures. Increases in maximal photosynthetic rates were not necessarily due to increases in algal biomass: chlorophyll-specific productivity increased in the presence of fish, reflecting in part changes in algal community structure. The relative contribution of picoplankton (0.2–2 μm) decreased in the fertilized enclosures (5%) relative to controls (33%) whereas that of nanoplankton (2–20 μm) increased with the addition of nutrients or fish, reaching almost 100% in the fertilized enclosures with fish added.

Résumé : Les effets d'un enrichissement en éléments nutritifs (N et P) et de la présence de poissons planctivores sur le rendement de conversion photosynthétique optimal et la répartition de la productivité primaire épilimnétique ont été mesurés dans des enceintes peu profondes (3,5 m) et profondes (11 m) aménagées dans un lac oligotrophe. Une stratification a été observée en été dans les enceintes profondes, l'épilimnion et la zone photique se situant aux mêmes profondeurs que dans les enceintes mixtes peu profondes. L'effet global de l'enrichissement en éléments nutritifs et de la présence de poissons a été identique dans les deux types d'enceintes. En présence ou en l'absence de poissons, la productivité du phytoplancton a presque décuplé dans les enceintes enrichies, tandis qu'elle a doublé en présence de poissons, tant dans les enceintes enrichies que dans les enceintes non enrichies. Les hausses du rendement de conversion photosynthétique optimal n'étaient pas nécessairement attribuables à une augmentation de la biomasse algale : la productivité spécifique de chlorophylle a augmenté en présence de poissons, reflétant les changements dans la structure de la communauté algale. La contribution relative du picoplancton (0,2–2 μm) était plus faible dans les enceintes enrichies (5%) que dans les enceintes témoins (33%), tandis que celle du nanoplancton (2–20 μm) était plus élevée dans les enceintes enrichies ou contenant des poissons, atteignant presque 100% dans les enceintes enrichies contenant des poissons.
[Traduit par la Rédaction]

Introduction

Although nutrients, especially phosphorus, can be used to predict algal biomass and productivity of lakes (e.g., Vollenweider 1978; Nicholls and Dillon 1978), there is substantial variability in productivity that cannot be explained by nutrient loading alone. For example, Schindler (1978) showed that in phosphorus-limited lakes, less than 50% of the variance in productivity could be explained by phosphorus loading. Some of this variance has been explained by predator-mediated changes in plankton community structure. The cascading trophic interactions hypothesis (Carpenter and Kitchell 1984; Carpenter et al. 1985), which attempts to explain such differences, states that top predators such as piscivorous fish can influence the abundance of planktivorous fish, which in turn can determine the abundance, size structure, and productivity of zooplankton and phytoplankton. The bottom-up and top-down model (McQueen et al. 1986, 1989) combines the influence of both predators (top-down) and resource (nutrient) availability (bottom-up). It predicts that top-down forces should be strong at the top of the food web and weaken towards the bottom, whereas bottom-up forces should be strong

Received October 26, 1994. Accepted December 7, 1995.
J12616

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at the lower trophic levels (algae) and weaken towards the top of the food chain (little or no influence of fish). It further implies that in eutrophic lakes, the impacts of changes at the top of food chain will dampen out as they cascade down through the food web so that there will be little or no influence on phytoplankton biomass and productivity.

From several experimental studies, increases in abundance of planktivorous fish appear to increase phytoplankton biomass (e.g., Hrbáček et al. 1961; Lynch and Shapiro 1981). Planktivorous fish can also influence algal community structure by altering the size distribution of algal biomass (Vanni 1987; Proulx et al. 1996). However, fewer studies (e.g., Carpenter et al. 1987; Sarvala and Jumppanen 1988) have included direct measurements of algal productivity, which is presumed to increase with planktivorous fish as well. Furthermore, while there is evidence that nutrient availability influences the size distribution of primary production (e.g., Stockner 1988), no studies have measured the impact of fish on the size distribution or quality of primary production. Since changes in nutrient availability as well as changes in zooplankton community structure can influence algal biomass and community structure (e.g., Watson and Kalff 1981; Vanni 1987; Hansson and Carpenter 1993), the relative importance of planktivorous fish in modulating primary production remains to be determined directly.

The purpose of this experiment was to determine the effects of planktivorous fish and nutrients as well as the interaction of these factors using large experimental enclosures in both shallow (3.5 m) and deep water (11 m) on the regulation of both the quantity and cell size distribution of primary production. Depth is a key variable in regulating sedimentation rates and production in lakes. In a stratified water column, nutrients are lost to the hypolimnion through sedimentation of particles, whereas in shallow systems with complete vertical mixing, nutrients would be continuously resuspended. On the other hand, the presence of planktivorous fish may reduce the sedimentation rates of nutrients in a stratified water column because reduced zooplankton grazing promotes the growth of small cells that sink slowly and are retained in the surface water (Mazumder et al. 1989). In general, shallow lakes tend to be more productive than deep lakes of similar trophic status (e.g., Pridmore et al. 1985). Furthermore, shallow systems may be more sensitive to planktivore predation because of the absence of zooplankton diurnal migrations and absence of refugia from predation. As a result, one would predict that the effects of planktivorous fish would differ in deep versus shallow systems.

Specifically we tested the following hypotheses: (*i*) the effects of fertilization and planktivorous fish on primary production are more pronounced in shallow water than in deep water, and (*ii*) the size distribution of the algae responsible for fixing carbon changes with fertilization and (or) presence of planktivorous fish.

Materials and methods

Study site and experimental design

The experiment was conducted during the summer of 1991 in 16 large enclosures (8 m in diameter each) at Lac Croche, a small (4.82 ha) oligotrophic lake situated at the Station de biologie des Laurentides de l'Université de Montréal, St. Hyppolite, Que. Lac Croche has a spring total phosphorus concentration of 5 $\mu g \cdot L^{-1}$. The epilimnion is typically 3 m deep with a summer temperature of 20–25°C and a photic zone (1% light level) of about 5 m. Eight enclosures were placed in shallow water at approximately 3.5 m and eight enclosures were placed in deeper water at approximately 11 m. The enclosures were constructed as described in Mazumder and Lean (1994) and were open to the sediments. The total volume was about 1.5×10^5 and 5.5×10^5 L for the shallow and the deep enclosures, respectively. All fish were initially removed from all enclosures by using a purse seine and prevented from re-entry with seine material fences (0.6 m). Throughout the experiment, minnow traps were used to ensure that the fish-free treatments remained free of fish and to determine fish growth rates and abundance in the fish enclosures. The deep enclosures stratified in summer with an epilimnion and photic zone of the same depth (3 m) as the shallow, mixed enclosures.

The experiment followed a two by two factorial design with duplicate enclosures for each treatment combination at each depth. The treatments were (*i*) control (C), no nutrients and no fish; (*ii*) no nutrient enrichment but with fish (F); (*iii*) enriched with nutrients but without fish (N); and (*iv*) enriched with nutrients and with fish added (N + F).

Nutrients were added weekly from 14 June to 15 October 1991 as a liquid mixture of sodium nitrate ($NaNO_3$) and phosphoric acid (H_3PO_4) combined in amounts to give a N:P ratio of 13:1 by weight (47.97 mg $N \cdot m^{-2} \cdot day^{-1}$ and 3.69 mg $P \cdot m^{-2} \cdot day^{-1}$) (Mazumder and Lean 1994). This loading rate was chosen to approximate the annual loading for a temperate eutrophic lake (Mazumder et al. 1988). Epilimnetic concentrations of total phosphorus ranged from 26 to 42 $\mu g \cdot L^{-1}$ in the enriched enclosures compared with 7–9 $\mu g \cdot L^{-1}$ in the untreated enclosures (Proulx et al. 1996).

On 12 June 1991, the F and N + F enclosures were each stocked with 150 hybrid redbelly dace (*Phoxinus eos* × *Phoxinus neogaeus*), resulting in a planktivore biomass of 5 $g \cdot m^{-2}$, similar to the planktivorous fish biomass of a moderately enriched lake. In more enriched lakes, the mass of planktivorous fish can be an order of magnitude higher (e.g., Christoffersen et al. 1993). All of the fish came from Lac Croche.

Primary productivity and size distribution

Photosynthetic carbon uptake was measured biweekly from 19 June 1991 until the end of August, for a total of six sampling dates. Duplicate water samples were taken from each enclosure using a 3-m integrated tube sampler. Primary production of phytoplankton was measured by the ^{14}C-bicarbonate method in duplicate 250-mL clear polycarbonate bottles (Lean and Burnison 1979). Bottles were incubated in Lac Croche from 11:00 to 13:00, at a depth where the light intensity was 250–300 $\mu E \cdot m^{-2} \cdot s^{-1}$ (1 $\mu E \cdot m^{-2} \cdot s^{-1}$ = 0.217 $W \cdot m^{-2}$). Thus, all samples were exposed to the same light regime. This light level was sufficient to achieve the maximal photosynthetic rate (P_{max}) as determined from experiments conducted in an incubator using different light intensities. The bottles were then retrieved and transported to the laboratory where samples were filtered within 2 h. Duplicate subsamples of water (generally 10 mL) were filtered through a 0.2-μm Nuclepore filter to determine total P_{max}. Other duplicate 10- and 50-mL subsamples were filtered through 2-μm Nuclepore and 20-μm Nitex filters separately to determine the distribution of P_{max} in three size-classes: (*i*) picoplankton (0.2–2 μm), (*ii*) nanoplankton (2–20 μm), and (*iii*) net plankton (>20 μm). Total radioactivity in each incubation bottle and the amount of radioactivity incorporated into the three size fractions were measured with a Beckman LS counter. Dissolved inorganic carbon for each enclosure was measured by gas chromatography (Stainton 1973) to calculate absolute carbon fixation rates.

Statistical analysis

The effects of nutrients, planktivorous fish, and depth on primary production and size distribution were assessed using a model I, three-

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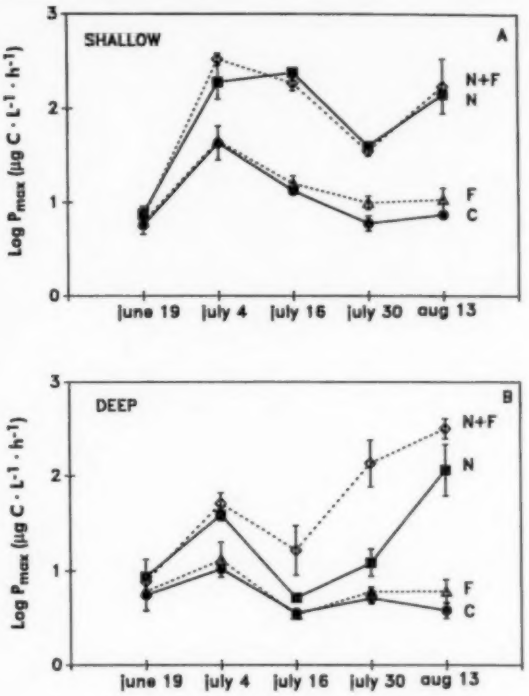
Fig. 1. Logarithm of maximal photosynthetic rate (P_{max}) for shallow enclosures (A) and deep enclosures (B) for control (C), fish addition (F), nutrient addition (N), and nutrient and fish addition (N + F) treatments.



way analysis of variance with repeated measures (ANOVAR). The analysis allows the use of several dates when comparing treatment effects, but does not assume that sampling dates are independent of each other (Winer 1971).

In this study, the between-subject effects were nutrients, fish, depth, and their related interactions (e.g., combined effects of fish and nutrients). The within-subject effects were time and all the interactions between time and the between-subject effects (e.g., combined effects of nutrients and time). Because all of the data satisfied the assumption of type H covariance, the usual F tests were used to test univariate hypotheses for the within-subject effects and associated interactions (SAS Institute Inc. 1985). The multi- and uni-variate tests gave similar results; therefore, only the results from the univariate F test are presented.

Three different ANOVARs were performed: one for testing total P_{max}, a second for the contribution of picoplankton to P_{max}, and a third for the contribution of nanoplankton to P_{max}. No statistical analyses were executed on the percent P_{max} of net plankton because the percentages for all treatments during all summer were very low (about ≤15%), making it difficult to test for significant effects. Data for total P_{max} were log transformed before ANOVAR was performed to reduce heterogeneity of variance. The distributions of percentages for pico- and nano-plankton were normalized by arcsine transformation before the ANOVARs were done (Zar 1984). The ANOVAR was run on the first five sampling dates for total P_{max} analysis and on all six sampling dates for percent P_{max} of pico- and nano-plankton. The last date of sampling was omitted for total P_{max} because the dissolved inorganic carbon measurements were missing for that particular date. Two simple three-way ANOVAs were performed to test the effects of nutrients, fish, and depth on chlorophyll *a* (chl *a*) concentration

Table 1. Repeated-measures ANOVA for the between-subject effects (nutrients (N), fish (F), depth (D), and their corresponding interactions) and within-subject effects (time (T) and related interactions on P_{max} (µg $C \cdot L^{-1} \cdot h^{-1}$)).

Source	df	P_{max}
Between subjects		
N	1	***
F	1	***
D	1	***
N × F	1	ns
N × D	1	ns
F × D	1	ns
N × F × D	1	*
Error	8	
Within subjects		
T	4	***
T × N	4	***
T × F	4	ns
T × D	4	***
T × N × F	4	ns
T × N × D	4	***
T × F × D	4	ns
T × N × F × D	4	ns

Note: Test for sphericity: $p = 0.571$, df = 9, $\alpha = 0.05$. Statistical significances are *, $p < 0.05$; ***, $p < 0.001$; ns, not significant ($p > 0.05$).

(micrograms chl *a* per litre) and on specific photosynthetic activity (total P_{max} per unit chl *a*) on 14 August.

The normality and the homoscedasticity of the residuals were tested by using the Wilk–Shapiro and Levene's tests, respectively, to meet the assumptions of ANOVA (SAS Institute Inc. 1985). All of the transformed data satisfied these assumptions ($p > 0.40$ for normality and $p > 0.30$ for homoscedasticity). All ANOVARs were run using the Statistical Analysis System (SAS) general linear models (GLM) procedure (SAS Institute Inc. 1985). A multiple comparison test, Tukey's test, was used to compare means for the effects of nutrients, fish, and depth at the 5% significance level on each sampling date.

From the ANOVARs, the power of ANOVA was performed if no significant effect from a factor was detected (Zar 1984).

Results

Total maximal photosynthetic rate

In the C and F enclosures, P_{max} ranged from 2 to 12 µg $C \cdot L^{-1} \cdot h^{-1}$ over the summer, whereas values over 100 µg $C \cdot L^{-1} \cdot h^{-1}$ were reached in the nutrient-enriched enclosures (Fig. 1). N + F enclosures attained the highest P_{max}, with values over 300 µg $C \cdot L^{-1} \cdot h^{-1}$ in midsummer for the shallow enclosures and in late summer for the deep ones. This represented about a twofold increase over the values in the N enclosures and a two order of magnitude increase over the controls. Overall, there were significant effects of nutrients (ANOVAR, $p < 0.001$), fish (ANOVAR, $p = 0.006$) and depth (ANOVAR, $p < 0.001$; Table 1).

Significant temporal variation occurred over the summer (Table 1). The trend for total P_{max} differed between fertilized and nonfertilized enclosures (ANOVAR, $p < 0.001$) and be-

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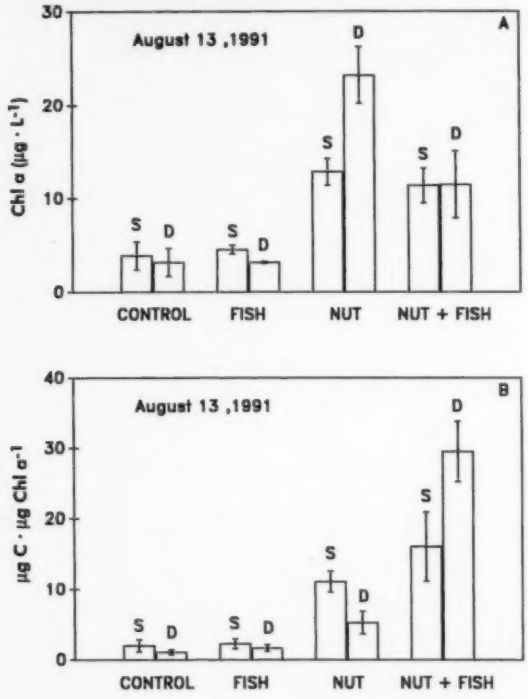
Fig. 2. (A) Chlorophyll *a* concentration ($\mu g \cdot L^{-1}$) of shallow enclosures (S) and deep enclosures (D) for control, fish addition, nutrient addition (NUT), and nutrient and fish addition (NUT + FISH) treatments on 13 August 1991. (B) Maximal chlorophyll specific photosynthetic rate (P_{max} : chl *a*) for both shallow enclosures (S) and deep enclosures (D) on 13 August 1991.



tween shallow and deep enclosures (ANOVAR, $p < 0.001$). Significant time–nutrient–depth (T × N × D) and nutrient–fish–depth (N × F × D) interactions were also detected (Table 1).

On 19 June, the first sampling date, no significant effects of nutrients, fish, and depth were detected. By early and mid-July, total P_{max} was 5–10 times higher in the shallow enclosures than in the deep enclosures, whereas later on there were no significant differences between the two depths (Fig. 1). There were no significant differences between the fishless and fish-added enclosures until the end of July during which the presence of fish doubled the total P_{max}. Nutrients increased total P_{max} by about sixfold for the whole month of July. However, in mid-August, total P_{max} increased dramatically (26-fold) in fertilized enclosures (N and N + F) compared with the nonfertilized ones (Fig. 1, C and F enclosures). The highest P_{max} values attained over the season in the shallow enclosures were similar to those measured in the deep enclosures.

In mid-August, chl *a* concentrations were similar between the N and N + F enclosures at both depths and were about four times higher (three-way ANOVA, $p < 0.01$) than in the C and F enclosures (Fig. 2A). Although for the experiment as a whole algal biomass was significantly affected by fish (Proulx et al. 1996), on this date chl *a* concentration was not affected by fish ($p = 0.24$) or depth ($p = 0.15$). The chlorophyll specific activity, that is the P_{max} per unit chl *a* for 13 August, was higher in the N + F enclosures and lower in the F and C enclosures (Fig. 2B). The N enclosures also had high chlorophyll-specific P_{max} but it was not as high as in the N + F enclosures. There were significant effects of nutrients (three-way ANOVA, $p < 0.01$) and fish ($p = 0.006$) on the chlorophyll-specific activity. These latter findings suggest that P_{max} was related not only to algal biomass but also to algal activity.

Table 2. Repeated-measures ANOVA for the between-subject effects (nutrients (N), fish (F), depth (D), and their corresponding interactions) and within-subject effects (time (T) and related interactions) on percent P_{max} of picoplankton (% pico) and nanoplankton (% nano).

Source	df	% pico	% nano
Between subjects			
N	1	***	***
F	1	ns	**
D	1	***	*
N × F	1	ns	**
N × D	1	*	ns
F × D	1	*	ns
N × F × D	1	ns	*
Error	8		
Within subjects			
T	5	***	***
T × N	5	**	***
T × F	5	ns	**
T × D	5	***	***
T × N × F	5	ns	*
T × N × D	5	***	**
T × F × D	5	ns	ns
T × N × F × D	5	ns	ns

Note: Test for sphericity: $p = 0.093$ (% pico), $p = 0.200$ (% nano), $\alpha = 0.05$. Statistical significances are *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant ($p > 0.05$).

Size distribution of maximal photosynthetic rate

Picoplankton (0.2–2 µm)

The contribution of picoplankton to P_{max} decreased with nutrient additions (ANOVAR, $p < 0.001$), with planktivorous fish having no influence (ANOVAR, $p = 0.30$, power of the test = 0.97). Temporal variation (ANOVAR, $p < 0.001$) occurred over the summer (Table 2). The trend for percent P_{max} of picoplankton differed between fertilized and nonfertilized enclosures (ANOVAR, $p = 0.005$) and between shallow and deep enclosures (ANOVAR, $p < 0.001$).

There were no significant effects of nutrients, fish, and depth on percent P_{max} of picoplankton at the start of the experiment (19 June), and the effect of fish remained nonsignificant throughout the summer. Following an initial increase (4 and 16 July), the contribution of picoplankton declined significantly (30 July – 28 August) with fertilization both in the shallow and in the deep enclosures until <10% of P_{max} was due to picoplankton (Fig. 3). In contrast, in the nonfertilized enclosures, the contribution to P_{max} remained between 25 and 45%, with fish causing a modest increase in the shallow and a decrease in the deep enclosures as the season progressed (Fig. 3). Depth was also found to be significant (ANOVAR, $p < 0.001$)

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Fig. 3. Percent maximal photosynthetic rate (P_{max}) for picoplankton shallow enclosures (A) and deep enclosures (B) for control (C), fish addition (F), nutrient addition (N), and nutrient and fish addition (N + F) treatments.

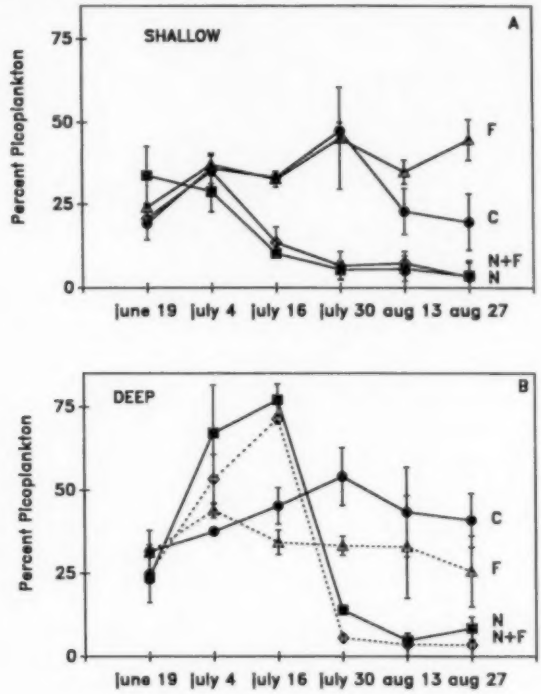
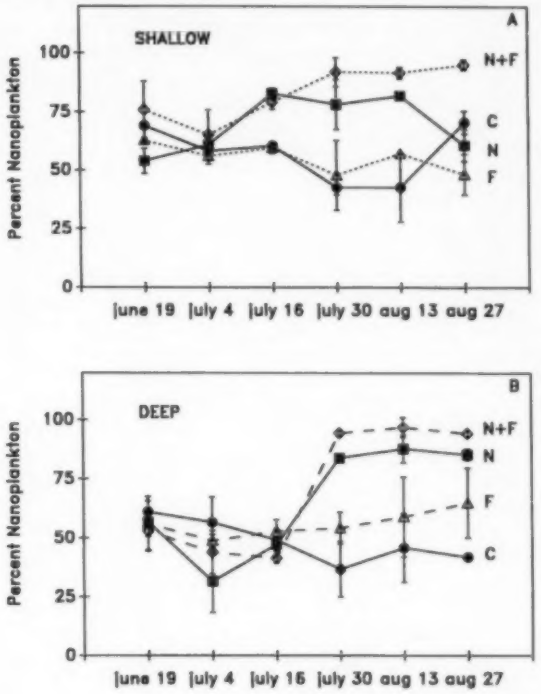


Fig. 4. Percent maximal photosynthetic rate (P_{max}) for nanoplankton in shallow enclosures (A) and deep enclosures (B) for control (C), fish addition (F), nutrient addition (N), and nutrient and fish addition (N + F) treatments.



at the beginning of July (4 and 16 July) when the percent P_{max} of picoplankton was two to three times higher in the deep enclosures than in the shallow ones.

T × N × D, N × D, and F × D interactions were detected as well (Table 2). In the deep fertilized enclosures the percentages increased in early and mid-July, whereas in the shallow fertilized enclosures the percentages decreased during that time. The percentages in the shallow F enclosures increased while the percentages decreased in the deep F enclosures.

Nanoplankton (2–20 μm)

Nanoplankton productivity changed significantly over time (Table 2); the trend for percent P_{max} of nanoplankton differed between fertilized and nonfertilized enclosures (ANOVAR, $p < 0.001$), between fishless and fish-added enclosures (ANOVAR, $p = 0.003$), and between shallow and deep enclosures (ANOVAR, $p < 0.001$).

The percent contribution of nanoplankton was similar (ca. 60%) in all treatments on the first two sampling dates (Fig. 4). However, the contribution of nanoplankton to P_{max} was 1.3–2 times higher in the shallow enclosures than the deep ones from 19 June to 16 July then became similar thereafter (ANOVAR, depth effect, $p = 0.016$; Table 2). In mid-July, the fertilized (N and N + F) enclosures increased by 1.3-fold over the nonfertilized (C and F) enclosures in shallow water (Fig. 4A), but all treatments were similar in deep water (Fig. 4B). From 30 July to the end of summer, the addition of nutrients increased productivity of nanoplankton by about 2 times and the addition of fish increased it by 1.5 times (ANOVAR, nutrient effect, $p < 0.001$; fish effect, $p = 0.002$; Table 2). P_{max} contributed by nanoplankton was lowest in the control followed by the fish-only and the nutrient-only enclosures. The percentages in the N + F enclosures reached almost 100% in late summer (Fig. 4).

Significant T × N × F ($p = 0.048$), T × N × D ($p = 0.004$), N × F ($p = 0.01$), and N × F × D ($p = 0.04$) interactions were also detected (Table 2). There were significant T × N × F and N × F interactions because enclosures with both nutrients and fish had higher percentages than enclosures with nutrients or fish alone for the last three sampling dates (Fig. 4). The T × N × D interaction was probably due to the fact that on 16 July the fertilized enclosures did not give similar percentages for both shallow and deep water (Fig. 4). One explanation for the N × F × D interaction is that on 27 August the enclosures with nutrients or fish alone gave lower percentages than the controls, whereas the enclosures with nutrients and fish together had the highest percentages in shallow water.

Net plankton (>20 μm)

The percent contribution of net plankton to P_{max} was very low in all four treatments at both depths throughout the summer (≤15%) and was almost 0% in the enclosures with nutrients and fish (N + F) during August.

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Discussion

Not surprisingly, nutrient enrichment (N and P) greatly increased primary production of phytoplankton, as estimated by the measurement of P_{max}, in both shallow and deep waters, with effects more pronounced towards the end of the summer (Fig. 1). However, the response to nutrient enrichment was not significantly greater in the shallow enclosures. This result was not anticipated given the literature documenting higher productivity of shallow versus deep systems. The reason for the lack of response was probably that very similar nutrient levels were attained in all nutrient-enriched enclosures, regardless of depth (Proulx et al. 1996). The sediments of this oligotrophic lake appeared to be a sink for nutrients such that nutrients did not accumulate over time in the water column of the shallow enclosures. The deep enclosures may in fact have had slightly higher areal production than the shallow ones since metalimnetic production was not directly measured. However, the poor light penetration in this dystrophic lake meant that metalimnetic production was a small component of the total production.

The hypothesis that the effects of nutrients and planktivorous fish together would be more pronounced in shallow water than in deep water was rejected. Although in early and mid-July the effects of nutrients and fish on total P_{max} were more pronounced in shallow water than in deep water, the effects were similar from 30 July until the end of the summer (Fig. 1).

Nevertheless, this experiment clearly demonstrated that planktivorous fish can have a significant effect on primary production, regardless of the depth of the system. The effect of both nutrients and planktivorous fish on productivity of phytoplankton was additive, that is the increase in total P_{max} in the N + F enclosures was greater than the combined increase in the planktivorous fish and nutrients treatments. That this enhanced carbon fixation was not just lost to respiration is supported by the high levels of particulate organic carbon measured in N + F treatments relative to the N treatments, particularly in the deep enclosures (Proulx et al. 1996).

The top-down and bottom-up theory (McQueen et al. 1989) predicts that in eutrophic conditions planktivorous fish will have little impact on phytoplankton productivity and biomass. This study, however, showed that the relative effects of planktivorous fish on total P_{max} were the same in nonfertilized and fertilized conditions. In August the presence of fish caused about a twofold increase in P_{max} in the fertilized enclosures, the same magnification as in the nonfertilized ones (Figs. 1 and 2).

The stimulatory effect of fish was observed even on days when there was no significant effect of fish on algal biomass as measured by chl *a* (Fig. 2). Overall, fish had a statistically significant effect on algal biomass as estimated from microscopic enumerations only in the deep enclosures (Proulx et al. 1996), even though there was a highly significant effect on productivity at both depths. The presence of planktivorous fish had significant effects on P_{max} independently of biomass probably because of changes in the phytoplankton biomass size distribution (Proulx et al. 1996) as well as the activity of phytoplankton (Fig. 2). For example, even though fish had no effect on algal biomass in the shallow enclosures, there was a significant effect on algal abundance (numbers per litre) because of a shift to small cells in the presence of fish (Proulx et al. 1996). Chlorophyll-specific productivity ($\mu g\ C \cdot \mu g\ chl\ a^{-1} \cdot h^{-1}$) was significantly higher under the N + F treatments, the combined result of a higher nutrient supply and the predominance of small cells that generally have higher photosynthetic activity than large cells (e.g., Desertova 1976; Malone 1980).

The changes in size distribution of algal production occurred mostly at the nanoplankton level (2–20 μm). In general the percentages contributed by nanoplankton to total productivity were always higher than those contributed by picoplankton (and net plankton) in all treatments throughout the summer (Figs. 3 and 4). The productivity of net plankton (less than 15% total productivity) was low in all of the treatments throughout the summer but particularly in the N + F treatments.

The percentage of P_{max} contributed by nanoplankton increased significantly with the presence of planktivorous fish in both fertilized and nonfertilized systems whereas that of picoplankton was not affected by fish (Table 2). In the nutrient and fish treatments, nanoplankton contributed almost 100% of total productivity relative to about 60% in the untreated enclosures (Fig. 4). Nanoplankton are considered the main size-class consumed by herbivorous zooplankton (e.g., Burns 1968; Porter 1972; Gliwicz 1975; Leibold 1989). From the cascading trophic interactions (Carpenter et al. 1985, 1991) and biomanipulation (Shapiro et al. 1975) hypotheses, predation by planktivorous fish on zooplankton alters the size structure of the zooplankton community, resulting in a zooplankton community dominated by small species (e.g., Hrbáček 1962; Brooks and Dodson 1965; Lynch 1979). Since small zooplankton have lower grazing rates than large zooplankton (e.g., Burns 1968; Knoechel and Holtby 1986), such a change in the zooplankton community could explain the increased productivity of nanoplankton. In contrast, picoplankton are too small to be directly controlled by crustacean grazers.

Other mechanisms such as the increase in nutrients released by more dominant small zooplankton (Lehman and Scavia 1982; Scavia et al. 1984) or the direct release of nutrients through fish egestion and excretion (Vanni and Findlay 1990; Schindler 1992) might also serve to stimulate nanoplankton production, since nutrient enrichment alone also stimulated nanoplankton production (Table 2).

While the percent contribution of nanoplankton increased with the addition of nutrients (Table 2, Fig. 4), the contribution of picoplankton decreased significantly with nutrient additions. Picoplankton production is typically highest in very oligotrophic systems (e.g., Stockner 1988). The decline in picoplankton production was probably a direct result of significant declines in their abundance in the nutrient-enriched enclosures (Tzaras 1994) as predicted by empirical models (Pick 1991).

In summary, the results of this study show that total phytoplankton productivity and its size distribution can be stimulated by both nutrients and planktivorous fish in shallow or deep (stratified) waters. Nutrients clearly increased the contribution of nanoplankton to total primary production while suppressing picoplankton production. However, planktivorous fish independently also increased phytoplankton productivity and the nanoplankton fraction. Furthermore, planktivorous fish can stimulate primary production in the absence of any effect on algal biomass.

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Acknowledgements

We thank the crew that maintained the Lac Croche enclosures in 1991 (P. Marchessault, D. Quinn, M. Trépanier) and the staff of the Station de biologie des Laurentides, St. Hippolyte, Que., for providing outstanding working conditions. Two anonymous reviewers helped streamline and focus the findings. This work was supported by a summer Natural Sciences and Engineering Research Council of Canada (NSERC) scholarship to S.P., NSERC grants to F.R.P. and A.M., and support from the National Water Research Institute, Environment Canada, to D.R.S.L.

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Sedimentation of algae: relationships with biomass and size distribution[1]

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Abstract: The influence of the biomass and size distribution of epilimnetic algae on patterns of algal sedimentation was determined in lake enclosures under contrasting conditions of planktivorous fish and nutrient loading (nitrate and phosphate). The addition of fish and nutrients increased the epilimnetic algal biomass, but the addition of nutrients increased the mean length of algae in fish-free enclosures and reduced the mean length in the enclosures to which fish had been added. Both the net (milligrams per square metre per day) and relative sedimentation rates (percent epilimnetic algal biomass per day) of algae were higher in the fishless than in the fish enclosures and were lower in the nonenriched than in the enriched enclosures. The consistently greater net and relative sedimentation rates in the fish-free than in the fish enclosures seemed to reflect a three to five times greater mean length of epilimnetic algae in the fish-free enclosures. Overall, both net and relative sedimentation were more closely related to the mean length than to the biomass of epilimnetic algae. Our results suggest that sedimentation cannot be assumed to be a constant fraction of biomass (a critical assumption in most mass-balance models for nutrients and contaminants) and that a relatively greater fraction of biomass is lost because of sedimentation as the mean length of algae increases.

Résumé : L'influence de la biomasse des algues épilimnétiques de même que la distribution de la biomasse selon un spectre de taille sur les patrons de la sédimentation phytoplanctonique ont été étudiées en enclos limnétiques soumis à des conditions contrastantes de planctivorie (présence–absence de poissons) et d'enrichissement en éléments nutritifs (présence–absence de fertilisation en nitrates et en phosphates). La biomasse épilimnétique des algues a augmenté aussi bien avec l'ajout de poissons qu'avec celui d'éléments nutritifs. Les éléments nutritifs ont été associés à une augmentation de la taille moyenne des algues dans les enclos sans poissons et à une baisse de la taille moyenne dans les enclos avec poissons. Les taux de sédimentation net (milligram par mètre carré par jour) et relatif (pourcentage de biomass épilimnétique des algues par jour) se sont avérés plus élevés dans les enclos sans poissons que dans les enclos avec poissons alors qu'ils étaient plus faibles dans les enclos non enrichis que dans ceux qui l'étaient. Le taux de sédimentation accru dans les enclos sans poissons semble être lié à la taille moyenne des algues épilimnétiques qui était de trois à cinq fois plus élevée dans les enclos sans poissons. Les taux de sédimentation net et relatif semblent plus apparentés à la taille moyenne des algues qu'à leur biomasse totale. Nos résultats suggèrent que dans les équations de bilans massiques de la dynamique des éléments nutritifs et des contaminants, on ne peut pas continuer à assumer que le taux de sédimentation est une fraction constante de la biomasse totale puisqu'une part accrue de la biomasse sédimente lorsque la taille moyenne des algues s'accroît.

Introduction

Algae are lost from the epilimnion of aquatic ecosystems via two major pathways: (*i*) grazing by zooplankton (Bergquist et al. 1985; Mazumder et al. 1990*a*, 1990*b*) and (*ii*) sedimentation to the hypolimnion (Reynolds et al. 1982*a*, 1982*b*; Sommer 1983, 1989; Bloesch and Uehlinger 1986; Baines et al. 1994). Although grazing by zooplankton on algae has received much attention and several models predicting zooplankton grazing have been published (Knoechel and Holtby 1986*a*, 1986*b*; Mazumder et al. 1990*b*), factors regulating algal sedimentation and patterns of algal sedimentation under contrasting structure of pelagic communities need further investigation (Mazumder et al. 1989). Most previous studies on algal sedimentation used standing crops of epilimnetic algae as a major determinant of sedimentation rates (Vollenweider 1968; Reynolds and Wiseman 1982; Reynolds 1983, 1984; Rees et al. 1991; Evans and Hakanson 1992; MacKay 1993). The size distribution of algae as a determinant of algal sedimentation has seldom been considered (Bürgi et al. 1979; Mazumder et al. 1989).

Determining the factors that influence the sedimentation of algae is fundamental because several areas of research in aquatic sciences, such as the seasonal succession of algae, cycling and mass balance of nutrients and contaminants, and paleolimnology (Moss 1980; O'Sullivan et al. 1984; Rees et al. 1991; O'Sullivan 1992), use sedimentation as an important variable. Most models describing the dynamics of algae (Bienfang 1981; Reynolds et al. 1982*a*, 1982*b*; Reynolds 1983, 1984; Sommer 1983, 1989), nutrients (Bienfang 1981; Tilman 1982; Reynolds 1984; Bloesch and Uehlinger 1986; McCauley et al. 1989), and contaminants (MacKay 1993) as-

Received June 9, 1994. Accepted December 7, 1995.
J12412

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sume that a constant fraction of algae, nutrients, or contaminants in the epilimnion is lost to the hypolimnion via sedimentation. On the contrary, others have suggested that the fraction of epilimnetic biomass that is lost because of sedimentation is quite variable among lake ecosystems and that the size distribution of epilimnetic algae is a potential factor determining algal sedimentation (Bürgi et al. 1979; Mazumder et al. 1989). However, these studies have been based on the size distribution of phosphorus or carbon in the epilimnion or on bulk estimates of sedimenting materials.

Models of the sinking patterns of algae predict that larger cells sink faster than smaller cells (Smayda and Boleyn 1965; Smetaceck 1980), while mass balance models assume that a constant fraction of epilimnetic biomass is lost via sedimentation (Vollenweider 1968; Evans and Hakanson 1992; MacKay 1993). The assumption that a constant fraction of epilimnetic biomass is lost via sedimentation would not appear to be consistent with the observations of significant variation in algal mean size among lake ecosystems of variable nutrient loading (Leibold 1990; Watson et al. 1992) and food-web structure (Mazumder et al. 1990*b*). These inconsistencies led us to test the following hypotheses: (*i*) the net sedimentation rate of algae is more closely related to algal size distribution than to algal biomass in the epilimnion, and (*ii*) relative sedimentation rate as measured by the fraction or percentage of epilimnetic algal biomass collected in sediment traps increases with increasing mean length of epilimnetic algae. The results of our study will contribute to the patterns of linkage between structure of pelagic communities and potential biological processes (Carpenter et al. 1986; Mazumder et al. 1992).

To test these hypotheses it was necessary to create contrasting biomass and size distributions of algae in the epilimnion but under similar physical conditions. For example, turbulence in the water column is an important factor influencing the sedimentation rate of certain algae (Gliwicz 1980). We created differences in the biomass and size distribution of epilimnetic algae by modifying nutrient loading and (or) zooplankton grazer communities in large lake enclosures, while we used the lake outside the enclosures as a reference for a natural system.

We chose to use large in situ enclosures as opposed to cross comparisons among lakes to test our hypotheses because (*i*) enclosures were of same surface area and morphometry, and therefore our sedimentation rate estimates were less influenced by variable turbulence than if lakes of different size and morphometry were used; (*ii*) the mean and maximum depths of the enclosures were constant, and therefore the distance between the epilimnion and the sediment traps was relatively constant in all of the enclosures; and (*iii*) all of the enclosures had stratified water columns with distinct hypolimnia during summer months.

Materials and methods

Experimental design

Experiments were conducted from May through October of 1991 and 1992 in Lac Croche at the Station de biologie des Laurentides de l'Université de Montréal. Lac Croche is a small (6 ha, 11.5 m maximum depth), slightly colored dimictic temperate lake. To create contrasting phytoplankton communities, we used eight large enclosures (8 m diameter, 11.5 m deep, thermally stratified and open to the sediments) in a 2 × 2 factorial design with planktivorous fish (northern redbelly dace, *Phoxinus eos*; 1.67 g wet weight per fish; 5 $g \cdot m^{-2}$) and nutrients ($NaNO_3$ and H_3PO_4 at loading rates of 13 $g\ N \cdot m^{-2} \cdot year^{-1}$ and 1 $g\ P \cdot m^{-2} \cdot year^{-1}$) as factors (Mazumder et al. 1988). Nutrients were added weekly to four of the eight enclosures. Fish were added to two of the four enriched and two of the four nonenriched enclosures. Our manipulations thus produced four treatments: control (nonenriched, lacking fish), +F (nonenriched, having fish), +N (enriched, lacking fish), and +NF (enriched, having fish). Each treatment had two replicate enclosures.

Installation of sediment traps

Sediment traps were built with Plexiglas cylinders (6.5 cm diameter and 40 cm long) with rubber stoppers sealing the bottoms. The height:diameter ratio of the traps of 6:1 was used to minimize resuspension (Bloesch and Burns 1980). Each sediment trap had a floatation collar to prevent tilting. Traps were installed in the hypolimnion with their tops 1.5 m above the sediment (10 m below the surface). A cord was tied at the bottom of each trap and the cord was tied to an anchor. The traps were retrieved by slowly bringing the anchor and the traps to the surface. There were two traps in each enclosure, i.e., four traps per treatment and two traps in the lake near the enclosures. Sediment trap materials were collected every 2 weeks during May through October of 1991 and 1992. Bloesch and Burns (1980) found that the degradation of material in the sediment traps was less than 10% during a 2-week period. To compromise between weekly (too many samples to count) and monthly (high degradation as well as the possibility of high temporal variation in epilimnetic biomass) collections, we emptied the sediment traps every 2 weeks.

After retrieval, the total volume of water in the sediment trap was reduced to 1 L by decanting surface water through a hole made at the 1-L level. One 100-mL sample from each of the traps was preserved with Lugol's iodine solution (1% final concentration) and was used for microscopic observation using an inverted microscope equipped with an image analyzer (Ultimage for McIntosh). One 5-mL subsample from each 100-mL Lugol's preserved sample was settled for 24 h in a settling chamber. the length and width of each species of phytoplankton were measured using the image analysis system. At least 400 cells were counted from each subsample (Lund et al. 1958; Edgar and Laird 1993). We counted only the intact algal cells, as indicated by the presence of Lugol's stain inside the cells. To make a homogeneous suspension of cells, we needed to shake the sample before taking subsamples for settling. This procedure often broke algal colonies into smaller colonies and individual cells and faecal materials into unrecognizable debris. Therefore, we decided to measure and count individual cells to estimate mean size and biomass of sedimented algae. The shaking of samples to create homogeneous suspension may also have released some undigested species of algae from faecal materials into suspension, and we may have included them in our enumeration. However, algae (gelatinous forms), which are often suggested to pass through zooplankton, contributed very little to the epilimnetic biomass in all of our treatments (Proulx et al. 1993). Mean lengths of phytoplankton communities were corrected for the relative contributions of all of the species. Biomass of sedimented algae (milligrams wet weight per square metre) was calculated using the phytoplankton counter system developed by Hamilton (1990). Areal estimates were calculated using the diameter of sediment traps and the depth of the water column above the sediment traps.

Net sedimentation and relative sedimentation rates were calculated as milligrams wet weight per square metre per day and percentage of epilimnetic biomass per day (on the basis of the mean biomass on trap deployment and retrieval days; details in Mazumder et al. 1989). It was important to estimate the relative sedimentation rate of epilimnetic biomass because it allowed us to compare the net sedimentation rate with the relative sedimentation rate under contrasting biomass and size distributions of epilimnetic algae. While the net

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Fig. 1. Epilimnetic algal biomass (g wet weight·m^{-2}) in four treatments. Error bars represent 1 SE of the means (n = 4; 2 traps per enclosure). Areal estimates are based on integrated samples from 0–3 m.

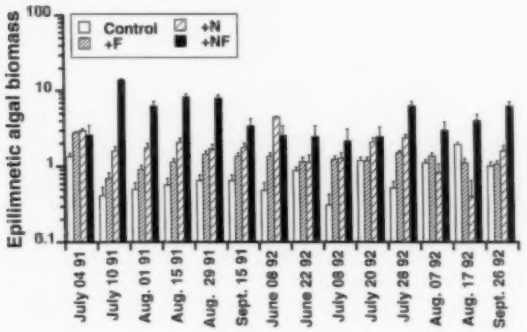

Table 1. Seasonal means for total phosphorus (TP, mg·m^{-2}; based on 0–3 m integrated sample), total chlorophyll concentration (Chl, mg·m^{-2}), total zooplankton biomass (TZB, µg·L^{-1}), zooplankton mean length (TZML, mm), *Daphnia* biomass (DB, µg·L^{-1}), and *Daphnia* mean length (DML, mm) in four treatments during May–September of 1991 and 1992.

		Control	+F	+N	+NF
TP	1991	37.5	35.7	139.5	155.9
	1992	41.7	56.4	192.0	494.1
Chl	1991	16.5	15.9	32.7	58.5
	1992	8.1	12.9	66.9	95.1
TZB	1991	3001.6	1243.1	5771.9	1101.3
	1992	4210.7	138.1	6494.5	371.4
TZML	1991	0.25	0.13	0.49	0.15
	1992	0.27	0.11	0.35	0.17
DB	1991	1173.8	33.7	2321.2	133.3
	1992	1794.9	66.7	2874.7	2.9
DML	1991	1.05	0.25	0.97	0.75
	1992	1.09	0.26	1.02	0.17

sedimentation rate of epilimnetic algae may increase with increasing epilimnetic biomass, the fraction of epilimnetic biomass that is lost as a result of sedimentation, i.e., the relative sedimentation rate of algae, may actually decline.

Integrated samples (0–3 m) were collected from the epilimnion with a tube sampler (6.5 cm diameter). These samples were used as indicators of the algal assemblages in the epilimnion. The same method as mentioned above for the enumeration of sediment trap algae was used to estimate the species composition, biomass, and size distribution of epilimnetic algal communities (Proulx et al. 1993).

Zooplankton samples were collected from 1, 3, 5, 7, and 9 m depths using a 35-L Patalas sampler and were counted using an image analysis system, which provided the biomass and size distribution of zooplankton. Summary data for zooplankton size distribution and biomass, nutrient concentration, and total chlorophyll concentration are presented to indicate contrasts in grazer communities, algal biomass, and trophic status (Table 1).

We used analysis of variance (ANOVA) with enrichment and fish addition as factors and two levels (with versus without fish and with versus without nutrient additions) for each factor. Seasonal means for each enclosure during each year (1991 and 1992, n = 16) were used for statistical analyses. Least-squares regressions were also used to examine the patterns of relationships between the biomass and size distribution of epilimnetic and sedimenting algae.

Table 2. Results of ANOVA showing the effects of fish additions, fertilization, and their interactions on log epilimnetic biomass of algae, log net sedimentation, ln relative sedimentation, and log mean length of epilimnetic and sediment trap algae.

Dependent variable	N effect	F effect	N × F interaction
Epilimnetic biomass	116	49	6
	(<0.001)	(<0.001)	(0.035)
Net sedimentation	78	60	3
	(<0.001)	(<0.001)	(0.102)
Relative sedimentation	2.8	388	0.40
	(0.120)	(<0.001)	(0.537)
Algal mean length (epi)	0.21	117	29
	(0.652)	(<0.001)	(<0.001)
Algal mean length (sed)	47	845	24
	(0.001)	(<0.001)	(0.001)

Note: F ratios and probabilities (in parentheses) are presented to indicate the significance of effects. n = 16 (seasonal means of each of the eight enclosures in 1991 and 1992; error degree of freedom is 12) for each of the variables.

Results

Biomass and size distribution of epilimnetic algae

Within 4 weeks after we started the experiments, the four treatments produced large contrasts in algal biomass in the epilimnion (Fig. 1). The control enclosures (no nutrient and fish additions) had abundant large *Daphnia* spp. (Tables 1 and 2) and exhibited the lowest algal biomass on 10 of 14 dates. The +NF enclosures (with nutrient and fish additions) lacked large *Daphnia* spp. and exhibited the highest algal biomass on 12 of 14 sampling dates. The nonenriched enclosures lacking large *Daphnia* spp. (+F) had higher algal biomass than the nonenriched enclosures having *Daphnia* spp. (control). On a seasonal average, algal biomasses were 0.70, 1.36, 2.02, and 4.33 g·m^{-2} in the control, +F, +N, and +NF enclosures during 1991, respectively. In 1992, the seasonal means were 0.95, 1.41, 1.99, and 4.18 g·m^{-2} in the four treatments, respectively. It is important to note the remarkable similarities in the response patterns of algal biomass to nutrient and fish additions between the 2 years (Table 1, Fig. 1).

The size distribution of algae as indicated by the mean length of algal cells was affected by nutrient and fish additions (Fig. 2). On a seasonal average the mean lengths of epilimnetic algae were 15, 7, 20, and 3 µm in control, +F, +N, and +NF enclosures, respectively, during 1991 (Fig. 2). The patterns of algal size distribution among treatments were similar during 1992. Among the fishless enclosures having large *Daphnia* spp. (control and +N), enrichment increased algal biomass as well as the mean length of algae, and a significant positive relationship (r^2 = 0.83, P = 0.002) between algal biomass and algal mean length was observed (Fig. 2). In contrast, among the fish enclosures lacking large *Daphnia* spp. (+F and +NF), enrichment increased algal biomass but the mean length of algae declined, and a significant negative relationship between algal biomass and the mean length of algae was produced (r^2 = 0.66, P = 0.015). Because of the contrasting responses of algal mean length to enrichment in the fish versus fishless enclo-

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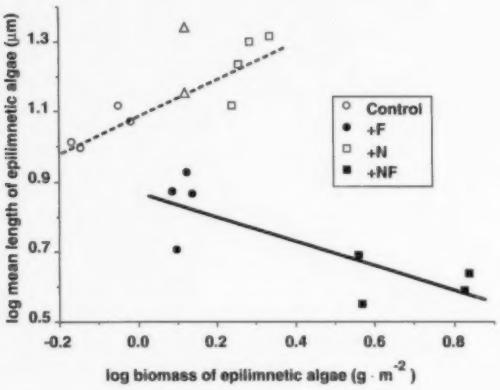
Fig. 2. Relationship between epilimnetic algal biomass and mean length (μm) of epilimnetic algae. Each data point is a seasonal mean for each of the eight enclosures during each of the 2 years. The broken line is the best linear fit for the open symbols (fishless enclosures having abundant large *Daphnia* spp., and the solid line is the best linear fit for the solid symbols (fish enclosures lacking large *Daphnia* spp.). Data points for the lake station (open triangles) are indicated for reference. Equations are presented in Table 3.

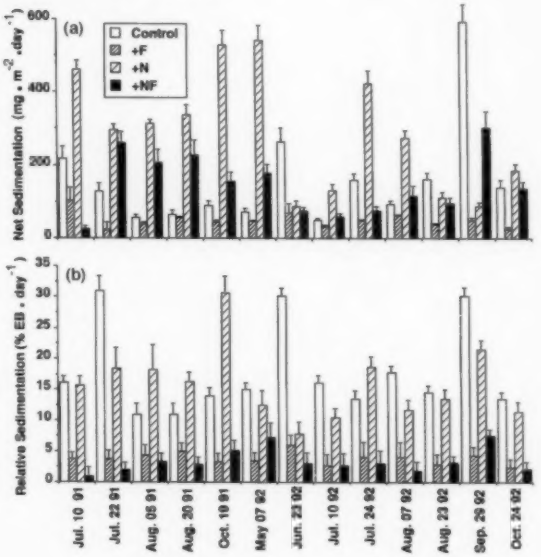


sures, ANOVA indicated a nonsignificant effect of enrichment and a highly significant interactive effect of enrichment and fish addition (Table 2). In the lake outside the enclosures, epilimnetic biomass was similar to that in the nonenriched fish enclosures, but the mean length of algae was close to that in the fishless enclosures (Fig. 2).

Patterns of algal sedimentation

On the basis of the collection of sediment trap materials on 13 dates during 1991 and 1992, we observed that the loss of algal biomass owing to sedimentation (net sedimentation) was significantly higher in the fishless enclosures (control and +N) than in the fish enclosures (+F and +NF) (Fig. 3*a*, Table 2). The nonenriched fishless enclosures (control) exhibited significantly higher ($P < 0.05$) net sedimentation rates than the nonenriched fish enclosures (+F) on 10 of 13 dates. The enriched fishless enclosures (+N) exhibited significantly higher net sedimentation rates than the enriched fish enclosures (+NF) on 9 of 13 dates. While there existed a clear pattern of increasing epilimnetic algal biomass from control to +F to +N to +NF treatments (Fig. 1), such a clear pattern was not exhibited by the net sedimentation data (Fig. 3*a*). Net sedimentation rates on average were 140, 49, 327, and 173 mg·m^{-2}·day^{-1} in control, +F, +N, and +NF enclosures in 1991, respectively. Similarly, they were 150, 52, 312, and 152 mg·m^{-2}·day^{-1} in control, +F, +N, and +NF enclosures, respectively, in 1992. When the net sedimentation rates were compared with the corresponding epilimnetic biomasses, an uncoupling between net sedimentation rate and epilimnetic biomass became obvious. The addition of fish was associated with a significant reduction in net sedimentation rate (ANOVA using seasonal averages, $P < 0.001$; Table 2), while the addition of fish was associated with a significant increase in the epilimnetic biomass ($P < 0.001$; Fig. 1, Table 2). Addition of nutrients was associated with higher net sedimentation rates in the +N enclosures than in the control enclosures and with a higher net sedimentation rate in the +NF enclosures than in the +F enclosures ($P < 0.001$). The highest net sedimentation rate was observed in enriched enclosures without fish, while the highest epilimnetic biomass was observed in the enriched enclosures with fish. The net sedimentation rate in the lake station near the enclosures was 200 mg·m^{-2}·day^{-1}, which was more comparable with the rate in the nonenriched fishless enclosures than any other treatments (Fig. 4).

Fig. 3. (*a*) Net sedimentation rates (mg·m^{-2}·day^{-1}) and (*b*) relative sedimentation rates (percentage of epilimnetic algal biomass sedimented per day) for the four treatments. Symbols and error bars are the same as in Fig. 1.



The relative sedimentation rate as indicated by the percent algal biomass sedimented per day was always greater in the fishless enclosures (nonenriched and enriched) having abundant large *Daphnia* spp. (control and +N) than in the fish enclosures lacking large *Daphnia* spp. (+F and +NF) (Fig. 3*b*). ANOVA (based on seasonal averages for each of the enclosures) indicated a significant effect of fish addition on relative sedimentation rate ($P < 0.001$; Table 2), whereas the addition of nutrients had no significant effect ($P = 0.400$). Overall, the relative sedimentation rate was three to four times greater in the nonenriched enclosures lacking fish (14–19% epilimnetic algal biomass (EB)·day^{-1} in control) than in the nonenriched enclosures having fish (4–4.1% EB·day^{-1} in +F). Among the enriched enclosures, the fishless enclosures having abundant large *Daphnia* spp. (14–16% EB·day^{-1} in +N) exhibited greater relative sedimentation rates than the fish enclosures lacking large *Daphnia* spp. (2.4–3.3% EB·day^{-1} in +NF) (Fig. 3*b*). In the lake, the relative sedimentation rates were as high as in the nonenriched and enriched fishless enclosures.

Relationships between algal biomass and sedimentation

For all of the enclosures taken together (seasonal means), the net sedimentation rate increased with increasing epilimnetic

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algal biomass (Fig. 4*a*), but the relationship explained only 10% of the variances ($r^2 = 0.10$, $n = 16$, $P = 0.225$). However, among the fish enclosures lacking large *Daphnia* spp. (+F and +NF) the relationship between epilimnetic algal biomass and net sedimentation rate was positive and highly significant ($r^2 = 0.90$, $n = 8$, $P < 0.001$; Table 3). Among the fishless enclosures having abundant large *Daphnia* spp., it was also positive and significant ($r^2 = 0.97$, $n = 8$, $P < 0.001$). The slopes of the epilimnetic biomass – net sedimentation rate relationships were similar for the fishless enclosures (0.96 ± 0.13) and the fish (0.83 ± 0.07) enclosures. In addition, neither of the slopes was significantly different from 1 ($P > 0.05$). However, the *y* intercept for the fishless enclosures (169 $mg \cdot m^{-2} \cdot day^{-1}$) was four times greater than that for the fish enclosures (41 $mg \cdot m^{-2} \cdot day^{-1}$; Table 3).

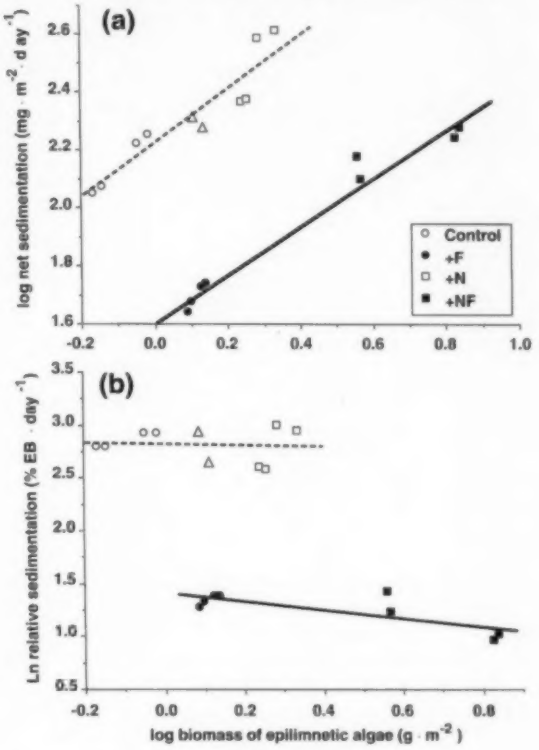
Overall, the relative sedimentation rate declined with increasing epilimnetic algal biomass (Fig. 4*b*). Although this relationship was significant ($P = 0.015$), it explained only 35% of the variances in relative sedimentation rate. Among the fishless enclosures having abundant large *Daphnia* spp., there was no relationship between epilimnetic algal biomass and relative sedimentation rate ($r^2 = 0.01$, $P = 0.810$). Among the fish enclosures lacking large *Daphnia* spp. the relationship was significant and negative ($r^2 = 0.57$, $n = 8$, $P = 0.030$).

Relationships between algal mean length and sedimentation

For all of the enclosures together, net sedimentation rate increased significantly with increasing mean length of epilimnetic algae ($r^2 = 0.25$, $n = 16$, $P = 0.050$) (Fig. 5*a*, Table 3). However, among the fishless enclosures having abundant large *Daphnia* spp. the relationship was also positive and highly significant ($r^2 = 0.92$, $n = 8$, $P < 0.001$), whereas among the fish enclosures lacking abundant large *Daphnia* spp. net sedimentation rate declined significantly with increasing mean length ($r^2 = 0.64$, $n = 8$, $P = 0.018$). In contrast to the weak positive relationship ($r^2 = 0.25$) between net sedimentation rate and mean length of epilimnetic algae (Fig. 5*a*), relative sedimentation rate increased significantly with increasing mean length of algae ($r^2 = 0.78$, $n = 16$, $P < 0.001$) (Fig. 5*b*, Table 3). Although all of the treatments together exhibited a significant increase in relative sedimentation rate, among the fishless enclosures having abundant large *Daphnia* spp. there was no significant increase in the relative sedimentation rate of algae with increasing mean length ($r^2 = 0.04$, $n = 8$, $P = 0.632$). The relationship was also positive but not significant among fish enclosures lacking large *Daphnia* spp. ($r^2 = 0.37$, $n = 8$, $P = 0.111$). However, if we compare the intercepts of the relationships for fishless versus fish enclosures, the intercept was several times greater in the fishless enclosures than in the fish enclosures (Fig. 5*b*, Table 3).

There was a significant positive relationship between the mean length of algae in the epilimnion and that collected in the sediment traps in 2 weeks (Fig. 6). The scatterplot of algal mean length in the epilimnion versus that in the sediment trap indicated a clear separation of the enclosures lacking large *Daphnia* spp. (+F and +NF) as well as the lake from the enclosures having large *Daphnia* spp. (control and +N). The scatterplot indicated that more than 75% of data points for the enclosures lacking large *Daphnia* spp. (+F and +NF) were below the 1:1 line, whereas 75% of the data points for the fishless

Fig. 4. (*a*) Relationship between epilimnetic algal biomass (EB) and net sedimentation rate. Data points and symbols are the same as in Fig. 2. (*b*) Relationship between epilimnetic biomass and relative sedimentation rate. Equations are presented in Table 3.



enclosures having large *Daphnia* spp. were above it. However, the slope of the linear fit through the data set for all of the enclosures was not significantly different from 1, suggesting that the overall size distribution of algae in the sediment traps was not statistically different from the epilimnetic size distribution of algae.

On average, sediment trap algae were 8 and 2.4 μm larger than epilimnetic algae in the fishless enclosures having abundant large *Daphnia* spp. (control and +N). In the fish enclosures lacking large *Daphnia* spp., sediment trap algae were on average 1 μm (+F) and 0.7 μm (+NF) smaller than epilimnetic algae in the fish enclosures lacking large *Daphnia* spp.. The greatest difference was observed in the control enclosures, where the sediment trap algae were 50% larger than the epilimnetic algae (Fig. 6). Outside the enclosures, the sediment trap algae were 1 μm larger than the epilimnetic algae.

Discussion

Both planktivorous fish predation and nutrient loading can modify the patterns of algal sedimentation rate by changing the biomass and size distribution of epilimnetic algae. However, the impacts of nutrient loading on the size distrbution of algae and associated patterns of algal sedimentation rate appear to reverse under contrasting conditions of large grazer commu-

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Table 3. Results of regression analyses showing the relative importance of biomass (log EB) and mean length (log EML) of epilimnetic algae in predicting net (log NS) and relative (ln RS) sedimentation.

Dependent variable	Independent variable	*y* intercept	Slope	*P*	r^2
NS (all data)	EB	2.06 ± 0.10	0.32 ± 0.25	0.225	0.10
NS (fishless)	EB	2.23 ± 0.03	0.96 ± 0.13	<0.001	0.90
NS (with fish)	EB	1.62 ± 0.03	0.83 ± 0.06	<0.001	0.97
NS (all data)	EML	1.57 ± 0.27	0.61 ± 0.28	0.050	0.25
NS (fishless)	EML	0.43 ± 0.23	1.65 ± 0.20	<0.001	0.92
NS (with fish)	EML	3.09 ± 0.36	−1.56 ± 0.48	0.018	0.64
NS (all data)	EB +	0.87 ± 0.22	0.80 ± 0.17	<0.001	0.73
	EML		1.14 ± 0.21	<0.001	
RS (all data)	EB	2.44 ± 0.22	−1.58 ± 0.57	0.015	0.35
RS (fishless)	EB	2.84 ± 0.07	−0.08 ± 0.31	0.810	0.01
RS (with fish)	EB	1.42 ± 0.07	−0.40 ± 0.14	0.030	0.57
RS (all data)	EML	−0.70 ± 0.40	2.93 ± 0.41	<0.001	0.78
RS (fishless)	EML	2.53 ± 0.60	0.26 ± 0.52	0.632	0.04
RS (with fish)	EML	0.72 ± 0.29	0.74 ± 0.40	0.111	0.37
RS (all data)	EB +	−0.31 ± 0.52	−0.45 ± 0.39	0.263	0.80
	EML		2.63 ± 0.48	<0.001	

Note: Seasonal means for each enclosure during each of 1991 and 1992 were used. $n = 16$ (seasonal means for each of eight enclosures in two seasons).

nities. Although both biomass and mean length of algae are important in determining algal sedimentation rate, algal mean length seemed to be a stronger determinant of sedimentation rate than algal biomass.

The addition of nutrients and (or) removal of large grazers by planktivorous fish created a 55-fold variation in the biomass (Fig. 1) and a 13-fold variation in the mean length (Fig. 2) of epilimnetic algae among the four treatments. These observations are consistent with previous studies that suggest that both reduction in grazing and addition of nutrient additions, individually or in combination, can increase algal biomass (Mazumder et al. 1988, 1992; Hansson 1992; Sarnelle 1992). However, our results also showed that in the fishless enclosures having abundant large grazers, the responses of epilimnetic algal biomass to nutrient additions are significantly weaker than what we would predict from nutrient loading or concentration alone (Mazumder and Lean 1994). This observation is consistent with patterns exhibited by data from whole-lake experiments as well as from unmanipulated lake ecosystems (Mazumder 1994*a*, 1994*b*). When large grazers (*Daphnia* spp.) were abundant, the addition of nutrients (nitrate and phosphate) increased both the biomass and mean length of epilimnetic algae. On the other hand, when large grazers were absent, the addition of nutrients increased algal biomass, but the mean length of algae decreased substantially. This divergent pattern of algal responses to nutrients under contrasting herbivory is different from existing models (Leibold 1990; Watson et al. 1992) that suggest that the contributions of large algae increase with increasing algal biomass regardless of grazer community structure. It needs to be noted that the extremes of algal biomasses achieved during our experiment are smaller than those used by Watson et al. (1992).

Among the nonenriched enclosures, the epilimnetic biomass was greater when the planktivorous fish removed the large grazers than when large grazers were abundant (Fig. 1), but the net sedimentation rate of epilimnetic algae was greater in the fish enclosures than in the fishless enclosures (Fig. 3*a*). Similarly, among the enriched enclosures, the epilimnetic biomass was greater in the fish enclosures lacking large grazers than in the fishless enclosures having abundant large grazers, but the net sedimentation rate was greater in the fishless enclosures than in the fish enclosures. If a constant fraction of the epilimnetic algal biomass was lost because of sedimentation (Vollenweider 1968; Bloesch and Uehlinger 1986; Uehlinger and Bloesch 1987; Evans and Hakanson 1992), then the net sedimentation rate should have increased proportionately with epilimnetic biomass and a strong relationship between epilimnetic biomass and net sedimentation rate should have been observed. Instead, a poor relationship between epilimnetic biomass and net sedimentation rate was observed (Fig. 4*a*). According to our first hypothesis, we expected a poor relationship between epilimnetic algal biomass and net sedimentation rate because we knew from previous experiments (Mazumder et al. 1988, 1992) that contrasting nutrient and fish additions not only change biomass of epilimnetic algae but also change their size distribution. Our results indicate that when the variation in mean length of algae is eliminated by considering enclosures of similar food-web structure (fishless and fish enclosures treated separately), highly significant positive relationships between biomass and net sedimentation rate are observed (Fig. 4, Table 3). However, the fishless enclosures with greater mean lengths of epilimnetic algae produced a relationship with a *y* intercept three times higher than that of the fish enclosures containing algae that were three to five times smaller. Such an elevated intercept for the fishless enclosures suggests that at similar epilimnetic biomass, ecosystems having large epilimnetic algae will have a greater sedimentation rate than ecosystems having a similar biomass but smaller algae.

In addition to the elevated sedimentation rate of algae in the fish-free enclosures relative to the fish enclosures, a poor relationship between algal biomass and net sedimentation rate might also occur because the percentage of epilimnetic biomass sedimented per day (relative sedimentation rate) was not a constant function of epilimnetic biomass. On average, 2–4% of the epilimnetic biomass sedimented per day in the

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Fig. 5. Relationships (*a*) between the mean length of epilimnetic algae and the net sedimentation rate and (*b*) between the mean length of epilimnetic algae and the relative sedimentation rate. Symbols are the same as in Figs. 2 and 4. Equations are in Table 3.

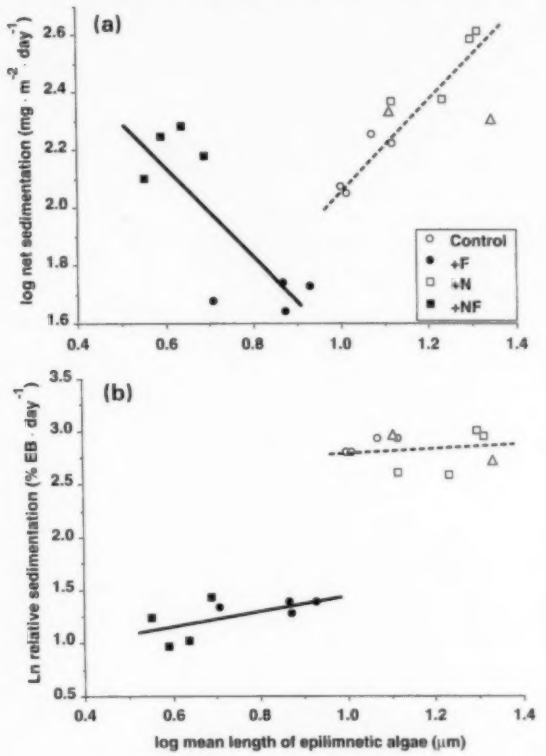
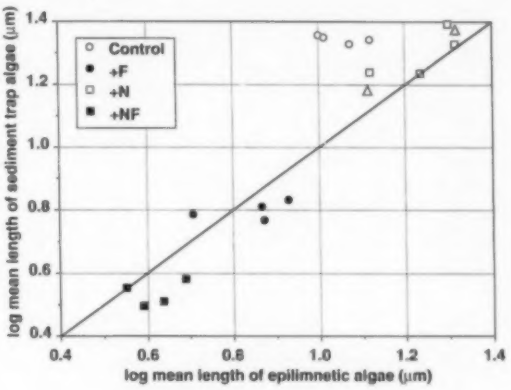


Fig. 6. Relationship between the mean length (μm) of algae in the epilimnion (means based on the data for the days of trap deployment and retrieval) and the mean length of intact algae remaining in the sediment traps on the day of trap collection. Symbols are the same as in Fig. 2. The diagonal line indicates the 1:1 relationship between epilimnetic mean length and sediment trap mean length.



fish enclosures compared with 14–16% in the fishless enclosures (Fig. 4*b*).

Results indicate that the size distribution of epilimnetic algae is an important determinant of the highly variable net and relative sedimentation rates among treatments. Overall, the relative sedimentation rate declined as the epilimnetic algal biomass increased (Fig. 4*b*). However, when the enclosures with and without fish were treated separately, no relationship between epilimnetic algal biomass and relative sedimentation rate was observed for fishless enclosures having abundant large *Daphnia* spp., but a significant negative relationship was observed for the fish enclosures lacking large *Daphnia* spp. The smaller mean length of algae may be responsible for the reduced relative sedimentation rate in the fish enclosures lacking large *Daphnia* spp.

That the size distribution of algae in the epilimnion is an important determinant of algal sedimentation is also indicated by the relationship between the mean length of epilimnetic algae and net as well as relative sedimentation rate (Figs. 5*a* and 5*b*). We expected the net sedimentation rate to increase significantly with increasing mean length of epilimnetic algae, but only 25% of the net sedimentation rate was explained by the mean length of algae (Fig. 5*a*, Table 3). Such a weak relationship between mean length and net sedimentation rate was produced by the contrasting patterns of relationships for fish versus fishless enclosures. In the fish enclosures, as the addition of nutrients increased algal biomass, their mean length declined, and a negative relationship between mean length and net sedimentation rate probably reflected greater differences in biomass between +F and +NF enclosures than the differences in the mean length. On the other hand, in the fishless enclosures, nutrient addition increased both the biomass and mean length of algae. As the increase in biomass and in mean length increased the sedimentation rate, a highly significant positive relationship between mean length and net sedimentation rate was observed for the fishless enclosures (Fig. 5*a*, Table 3). Previous studies predicted faster sedimentation for larger algal cells (Smayda and Boleyn 1965; Smetaceck 1980; Reynolds and Wiseman 1982).

Although the net sedimentation rate increased weakly with increasing mean length of epilimnetic algae (Fig. 5*a*), the relative sedimentation rate of algae increased significantly with increasing mean length (Fig. 5*b*). Several previous studies have suggested that the sinking rates of algae should increase with increasing mean size of algal cells (Reynolds and Wiseman 1982; Smayda 1970; Smayda and Boleyn 1965). However, among enclosures of similar food-web structure, i.e., among the enclosures lacking large *Daphnia* spp. or having large *Daphnia* spp., there was no significant relationship between mean length of epilimnetic algae and their relative sedimentation rate (Fig. 5*b*). The lack of a large gradient in mean length among enclosures lacking large *Daphnia* spp. (4-fold in +F and +NF) and among enclosures having large *Daphnia* spp. (6.5-fold in control and +N) may be responsible for the lack of a significant relationship under similar food-web structure. On the other hand, if the *y* intercepts of the two relationships are considered, the average relative sedimentation rate is seven times greater for the enclosures having abundant large *Daphnia* spp. than for the enclosures lacking large *Daphnia* spp. This significant increase in relative sedimentation rate with increasing mean length of algae was produced by a relative sedimentation rate of epilimnetic algae in the enclosures

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having abundant large *Daphnia* spp. (2–5 times greater in mean length) that was 3.5–6.5 times faster than in the enclosures lacking large *Daphnia* spp. This observation is not consistent with previous studies that suggest that a constant fraction of epilimnetic algal biomass is lost because of sedimentation (Bürgi et al. 1979; Bloesch and Uehlinger 1986; Uehlinger and Bloesch 1987; Evans and Hakanson 1992) whereas it is consistent with the work of other investigators who suggested that the loss of algae owing to sedimentation should also be dependent on algal size (Smayda and Boleyn 1965; Smayda 1970; Reynolds and Wiseman 1982; Mazumder et al. 1989).

That the mean length of epilimnetic algae is an important determinant of algal sedimentation is also supported by the observation that the addition of mean length of algae as a second variable to the epilimnetic biomass – net sedimentation rate relationship increased r^2 from 0.10 to 0.73 (Table 3). Similarly, addition of mean length to the biomass – relative sedimentation rate relationship also increased r^2 from 0.35 to 0.80. However, the variable species composition of epilimnetic algae in different treatments as well as the temporal changes in viscosity of the water column may also be important determinants of the variable loss rates of algae under contrasting nutrient loading and grazer communities. Furthermore, the contribution of metalimnetic algae to sedimentation may also be an important factor in determining algal sedimentation (Baines and Pace 1994), an aspect we were not able to evaluate in this study. Nevertheless, the biomass and size distribution of epilimnetic algae explained 80% of the variance in the relative sedimentation rate of algae, and the size distribution of algae in the epilimnion was very closely related to the sediment trap algae, suggesting that the metalimnetic contribution may not be as important as the epilimnetic contribution to sedimentation in our enclosures. Baines and Pace (1994) used bulk biomass (chlorophyll *a*; chl *a*) in the epilimnion and metalimnion to predict algal sedimentation and found that the metalimnetic chl *a* concentration was more closely related to sinking flux than epilimnetic chl *a* concentration. Their study could not demonstrate whether the metalimnetic chl *a* had a different species composition than the epilimnetic chl *a*. It is possible that in some lakes the metalimnetic chl *a* maximum is actually produced by the accumulation of sedimenting epilimnetic algae at the thermocline (density barrier), an aspect that needs to addressed in future studies.

There was a significant positive relationship between the mean length of algae in the epilimnion and that of algae collected in the sediment traps in 2 weeks (Fig. 6). The scatterplot of algal mean length in the epilimnion versus that in the sediment trap indicated a clear separation of the enclosures lacking large *Daphnia* spp. (+F and +NF) as well as of the lake from the enclosures having large *Daphnia* spp. (control and +N). If smaller algal cells sediment more slowly than larger cells, then there should be more large cells in the sediment traps than in the epilimnion as the mean size of epilimnetic algae increases. Consequently, the relationship between the mean lengths of epilimnetic versus sediment trap algae should produce a pattern with a slope greater than 1. The scatterplot indicates that six of eight data points for the enclosures lacking large *Daphnia* spp. (+F and +NF) are below the 1:1 line, and six of eight data points for enclosures having large *Daphnia* spp. are above it. However, the slope of the linear fit through the data set for enclosures was not significantly different from 1, suggesting that the overall size distribution of algae in the sediment traps was not statistically different from the epilimnetic size distribution of algae. A smaller mean length in the sediment trap than that in the epilimnion of enclosures lacking large *Daphnia* spp. might mean that smaller cells have a greater tendency to remain in the epilimnion than to sediment to the hypolimnion. A larger mean length of algal cells in sediment traps than that in the epilimnion, as is the case for enclosures having large *Daphnia* spp., might mean a proportionately greater contribution of larger cells in sediment traps than in the epilimnion. It is possible that larger metalimnetic algal species in these clearwater enclosures contributed to the sediment traps (Hurley and Garrison 1993; Baines and Pace 1994). The above relationships (Fig. 6) were based on mean lengths of algal cells, and they may or may not hold if the size distribution is expressed as mean cell volume instead of mean cell length. Given the same mean length, different species of algae may have a different volume per cell, which is an equally important parameter determining algal sedimentation (I. Larocque, A. Mazumder, M. Proulx, and D.R.S. Lean, unpublished data).

In conclusion, our results show that the sedimentation of algae is more closely related to the size distribution of algae than to the total standing crop of algae in the epilimnion. We have demonstrated, within the scope of the experimental enclosures, that algal sedimentation is not a constant function of epilimnetic algal biomass and that it varies strongly with changes in the size distribution of epilimnetic algae. Net and relative sedimentation rates outside the enclosures were similar to the patterns observed in the control and +N enclosures. In addition, most of the lake data for the size distribution of algae in the epilimnion versus that in the sediment traps fit a 1:1 relationship (Fig. 6). We believe that our results from experimental enclosures closely mimicked sedimentation in natural lakes. Patterns of relationships between the biomass and size distribution of epilimnetic versus sediment trap algae observed under contrasting conditions of enrichment and zooplankton grazers in the enclosures are convincing enough to warrant the development of a size-based model of sedimentation for natural lakes. Such models will have important implications for mass balance models for nutrients and contaminants as well as for models in paleolimnology. Finally, the resilience of ecosystems dominated by large herbivores to eutrophication, regardless of high nutrient loading, may be due to the fact that large grazers, by selectively grazing the small algae (Mazumder et al. 1988, 1992), give a competitive advantage to large algae, which tend to grow more slowly and sediment more quickly and prevent a high algal biomass accumulation in the epilimnion. However, this hypothesis may not apply to situations in which large algae with special adaptations for buoyancy are dominant, as is the case for filamentous blue-green algae.

For epilimnetic algal biomass to increase, the overall rate of loss of epilimnetic algae to grazing and sedimentation has to be slower than the growth rate. Using large enclosures, we produced extreme contrasts in the biomass and size distribution of large grazers as well as that of algae in the epilimnia. These contrasts in the biomass and size distribution of epilimnetic algae modified the patterns of algal sedimentation. Our previous experiments indicated that the loss of algae owing to

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grazing is substantially reduced when large grazers are eliminated by planktivorous fish (Mazumder et al. 1990*b*). The contrasting size distribution and biomass of zooplankton, especially that of *Daphnia* spp. among four treatments (Table 1), would suggest reduced loss of epilimnetic algae to grazers in the two treatments lacking abundant large *Daphnia* spp. The loss of algae owing to sedimentation was also reduced when grazer impact was less important (Figs. 3 and 4). Therefore, an excessive accumulation of algae in the epilimnion may occur when the losses of epilimnetic algae owing to both grazing and sedimentation are reduced.

Acknowledgements

This project was supported by Natural Sciences and Engineering Research Council (NSERC) grants to A.M., D.R.S.L., and F.R.P.; individual and group grants from Fonds pour la formation de chercheurs et l'aide à la recherche (Quebec) to A.M.; and an NSERC strategic grant to A.M. and D.R.S.L. We thank the staff at the Station de biologie des Laurentides de l'Université de Montréal for providing excellent research facilities. Thanks are also due to over 15 undergraduate research assistants for their assistance during fieldwork. Discussions with Stephen Baines, Michael Pace, John Downing, Bill Taylor, and Donn Branstrator and suggestions from anonymous reviewers helped to organize the manuscript.

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Arrival, spread, and early dynamics of a zebra mussel (*Dreissena polymorpha*) population in the Hudson River estuary

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Abstract: The zebra mussel (*Dreissena polymorpha*) population of the Hudson River estuary grew and spread rapidly following its first detection in May 1991. The population reached 550 billion animals (4000/m^2, mean over the freshwater tidal river) by the end of 1992, constituting >70% of zoobenthic biomass, and filtered a volume equivalent to the entire water column in 1 day. Over 95% of the population lived on subtidal rocks. Following the period of rapid population growth in 1991–1992, reproductive success (young of the year per adult) fell by four orders of magnitude in 1993–1994. Furthermore, at the end of the 1993–1994 growing seasons, young of the year were only 20–30% as large as in 1991. Adult zebra mussels may have been outcompeting the larvae for food. We propose that such food-limited zebra mussel populations may be especially frequent in rivers and estuaries, where the ratio of food supply to available substratum is small.

Résumé : Les effectifs de la moule zébrée (*Dreissena polymorpha*) dans l'estuaire de l'Hudson ont augmenté et se sont propagés rapidement depuis la découverte des premiers individus en mai 1991. La population s'établissait à 550 milliards de moules (4000 moules/m^2, moyenne établie pour la portion dulcicole de la rivière qui est soumise aux marées) à la fin de 1992. Elle formait alors plus de 70% de la biomasse zoobenthique et filtrait quotidiennement un volume équivalent à toute la colonne d'eau. Plus de 95% des moules vivaient fixées aux pierres de la zone subtidale. Par suite de l'explosion démographique enregistrée en 1991–1992, le succès de la reproduction (nombre de jeunes de l'année par adulte) a chuté de quatre ordres de grandeur en 1993–1994. En outre, la taille des jeunes de l'année à la fin de la saison de développement de 1993–1994 atteignait seulement 20 à 30% de celle des jeunes de l'année de 1991. L'incapacité des larves de résister à la forte compétition pour la nourriture exercée par les adultes pourrait avoir été la cause de ce phénomène. Les auteurs estiment que de telles populations limitées par la nourriture pourraient être particulièrement fréquentes dans les rivières et les estuaires, où la quantité de nourriture par rapport à la surface des supports est faible.
[Traduit par la Rédaction]

Introduction

Despite a long history of European research (summarized by Stanczykowska 1977; Mackie et al. 1988) and recent North American studies (e.g., Haag et al. 1993; Nalepa and Schloesser 1993; Nicholls and Hopkins 1993; Mellina and Rasmussen 1994; Ricciardi et al. 1995), many critical questions remain about the dynamics, control, and ecological effects of zebra mussel (*Dreissena polymorpha*) populations. Specifically, little is known about the dynamics and ecological effects of zebra mussel populations in habitats other than lakes or about the ecology of zebra mussels over long time scales or large spatial scales. Here, we describe the invasion and early population dynamics of the zebra mussel in the Hudson River estuary, as part of a comprehensive assessment of the ecological effects of the zebra mussel on this ecosystem. As we will show below, the zebra mussel population expanded very rapidly in the Hudson and has now apparently reached a self-limiting phase in which larvae are strongly suppressed by the adults. A companion paper (Caraco et al. 1996) shows that the zebra mussel has had enormous impacts on the Hudson's phytoplankton that differ in important ways from what has been described for lakes.

Our study focused on the freshwater part of the Hudson River estuary, extending from the dam at Troy, New York, (river kilometre (RKM) 248; i.e., 248 km upriver from the southern end of Manhattan in New York City) to Newburgh, New York (RKM 99). The study area covers 140 km^2 and has a mean depth of 8.3 m (Gladden et al. 1988). The entire study reach is subject to tides of 0.8–1.6 m and is well mixed vertically by tidal flows. Water in the Hudson is hard (Ca^{2+} concentration = 22–30 mg/L), nutrient rich (NO_3-N concentration = 0.5–1 mg/L; soluble reactive P concentration = 3–30 µg/L), and moderately turbid (Secchi disk depth = 1–2 m; concentration of suspended solids = ~20 mg/L) (Cole et al. 1991, 1992; Findlay et al. 1991*a*, 1991*b*). The sediments are dominated by various mixes of sand, silt, and clay, but about 7% of the bottom is rocky (Simpson et al. 1986; D.L. Strayer, J. Powell, P. Ambrose, L.C. Smith, M.L. Pace, and D.T. Fischer, unpublished data). The Mohawk River enters the Hudson just above the head of the estuary. The Mohawk is part of the New York State Barge Canal, which connects the Hudson with lakes Erie

Received May 8, 1995. Accepted November 20, 1995.
J12910

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and Ontario and has been seen as a potential invasion route for the zebra mussel (e.g., Griffiths et al. 1991).

Methods

To estimate the size of the zebra mussel population in the Hudson, we subdivided the freshwater estuary into nine sampling strata, representing three habitats (rocks, fine-grained sediments, and unionid clams) in each of three geographic strata (RKM 99–151, RKM 151–213, RKM 213–248). We estimated the extent of rocky versus fine-grained (i.e., sand–silt–clay) sediments by taking petite PONAR grabs (15 × 15 cm) at 253 sites throughout the estuary. If we failed to retrieve a sample in repeated (more than five) grabs, we classified the site as rocky; otherwise, we classified the site as fine grained.

Zebra mussel densities on rocks were estimated in two ways. In 1991–1992, we took rock samples using a modified oyster dredge from four (1991) or nine (1992) sites in the infested area of the river. We counted the number of zebra mussels on each rock and estimated rock area by covering rocks with a layer of aluminum foil and weighing the foil. Surface areas measured in this way were converted to square metres of river bottom occupied by the rock by dividing by 3 (cf. Bailey et al. 1995). In 1993–1994, divers collected at least 10 rocks from each of six sites in the river (two in each geographic stratum). We counted the number of zebra mussels per rock and estimated the area of the river bottom occupied by the rock as the maximal projected area of the rock. In 1992–1994, we measured the shell lengths (and dry masses, see below) of subsamples of zebra mussels from each rock.

To estimate the number of zebra mussels on fine-grained sediments and unionids, we took samples from 16 sites in each of the three geographic strata. Five samples were collected from each site with a standard (22.9 × 22.9 cm) PONAR grab and sieved in the field through a 2.8-mm mesh. Each unionid in the sample was bagged separately and the sieve residue frozen. Zebra mussels and unionids were removed from thawed samples in the laboratory and counted.

To follow dynamics of zebra mussel populations on rocky shorelines, we took monthly samples from a rocky beach on Cruger Island (RKM 158). Five or 10 rocks were taken from each of three elevations: 34 cm above mean low tide, mean low tide, and 34 cm below mean low tide. The maximal projected area and the number of zebra mussels on each rock were recorded.

We estimated the numbers and size of young-of-the-year (YOY) zebra mussels by sampling navigation buoys in 1991–1994. In December or January, the U.S. Coast Guard replaces summer navigation buoys in the Hudson with winter buoys. About 40 of these buoys are changed in the freshwater tidal reach of the Hudson. Because these buoys are stored in a yard over the winter, there is no carry-over of mussels from year to year. We estimated the number of YOY per metre circumference of the buoy (we used circumference rather than area because most zebra mussels settled in a band along a seam near the base of the buoys) as an index of year-class strength and removed a sample of 10 mussels from each buoy for later measurement of shell length.

Veligers were sampled biweekly during the ice-free season at Kingston, New York, by pumping 42 L of surface water through a 73-μm mesh plankton net. In 1992–1993, samples were counted live upon arrival in the laboratory. In 1994, samples were preserved in buffered formalin and counted using cross-polarized light (Marsden 1992). All counts were made with a stereomicroscope at magnifications of 25× or greater.

To estimate the biomass of the zebra mussel population, we regressed dry body mass (dried overnight at 60°C) on shell length and applied these equations to shell lengths and densities observed in the field.

To estimate zebra mussel filtration rates in the field, we applied the regression of Kryger and Riisgard (1988) to observed numbers and sizes of zebra mussels in the Hudson River. Our own estimates of filtration rates of zebra mussels on natural Hudson River water agree closely with Kryger and Riisgard's results (Roditi et al. 1996). (We did not use the results of Roditi et al. here because they are not normalized for body size of zebra mussels.) All estimates of filtration rates presented here are based on 20°C.

We roughly estimated the food demands (assimilation) of the zebra mussel population by summing estimates of respiration and production. To estimate respiration, we fitted a regression through the laboratory data of Dorgelo and Smeenk (1988), Sprung and Borcherding (1991, day 2 data), and Quigley et al. (1993), producing the following general equation:

$$R = 61.9\mathrm{DM}^{-0.4653} \times 10^{0.01208\,T}$$

where R is respiration (milligrams O_2 per gram dry matter per day), DM is dry body mass (milligrams), and T is water temperature (°C) (for the log-log regression, adjusted $r^2 = 0.92$, $n = 11$, $p < 0.0001$). We estimated respiration from this equation using observed body sizes and water temperatures from the Hudson River, assuming respiration was negligible before 3 April and after 2 December, when water temperatures were low (<4°C). We estimated production in two ways: from respiration, assuming a net growth efficiency of 30% (cf. Valiela 1984), and from biomass, assuming an annual production to biomass ratio of 1 (Stanczykowska 1976; Waters 1977; Cleven and Frenzel 1993).

Some of the confidence intervals we present would have been difficult or impossible to calculate using conventional statistical methods and were estimated instead by bootstrap methods using Resampling Stats (Bruce 1993) with 1000 runs.

Results and discussion

Chronology of the invasion

Zebra mussels were first seen in the Hudson near Catskill in May 1991 (Fig. 1). By the end of 1991, zebra mussels had spread downriver 80 km to Newburgh, but just one animal was seen more than 5 km upriver of Catskill, despite extensive searches of the upper estuary (Fig. 1). Only 1 year later, zebra mussels were found everywhere that we sampled in the freshwater and oligohaline parts of the estuary (Fig. 1), reaching as far downriver as northern Haverstraw Bay, where salinity during the settling season was 3–5 ppt (Walton 1993). The range in the estuary has not expanded since 1992 and presumably now is limited by salinity.

The pattern of spread shows that the Hudson received two distinct invasions. The first invasion came near Catskill from some unknown human vector (e.g., barges, boat trailers) and an unknown source, probably in 1989 or 1990. The population must have been large by the end of 1990 (at least ~10^6 animals; cf. Fig. 2), so the initial inoculum would have had to have been very large if it came in 1990. Alternatively, the population may have been building from a small inoculum that came no later than 1989. The initial invasion of the Hudson represented a large jump across country by the zebra mussel, as the nearest potential source populations were in Lake Erie in 1989 and Lake Ontario and the western part of the Erie Canal in 1990 (O'Neill and Dextrase 1994). A second invasion occurred in 1992, when zebra mussels were carried into the head of the estuary by fluvial transport from the Mohawk River. (The Mohawk itself was not infested until 1991 (O'Neill and Dextrase 1994).) The two populations merged in 1992.

Growth of the population and its activity

The spread of zebra mussels in the Hudson was accompanied

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Fig. 1. Spread of zebra mussels through the Hudson River estuary, 1991–1992. Solid circles show sites where zebra mussels were found, and open circles show sites where zebra mussels were sought but not found. From left to right: September 1990 through June 1991, September through December 1991, and September through December 1992.

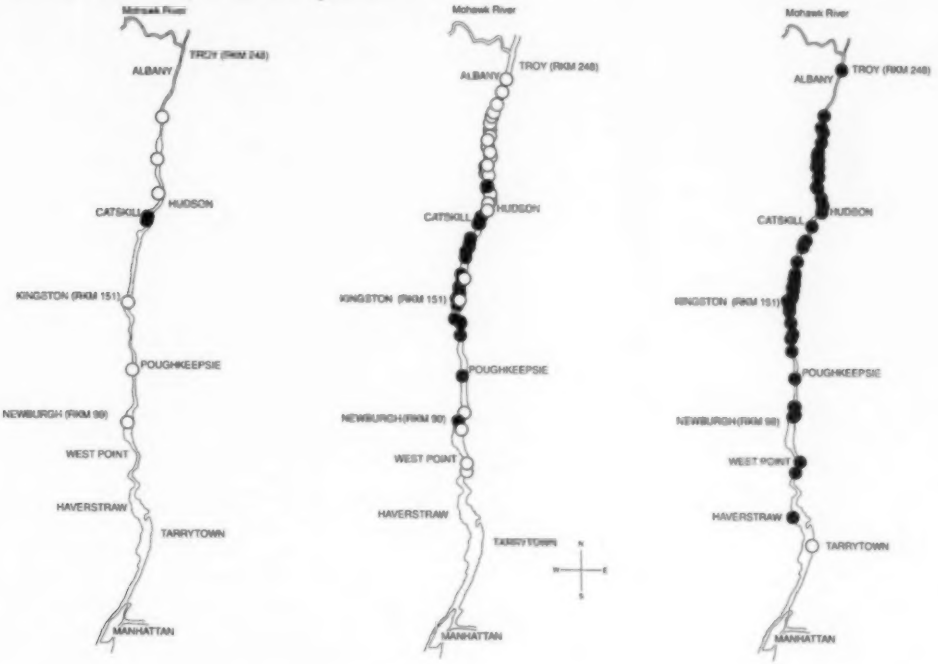
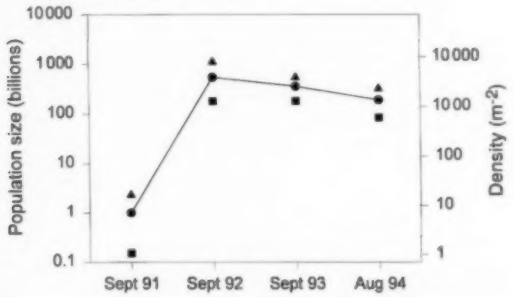


Fig. 2. Growth of the zebra mussel population in the Hudson River estuary, 1991–1994, expressed both as total population size and as mean density over the entire study area (RKM 99–248). Shown are the means (line) and 95% confidence limits (squares and triangles), as estimated by bootstrap methods.



by rapid growth in the size and activity of the population (Fig. 2, Table 1). By the end of 1992, the population had reached 550 billion individuals and a biomass of 28 g DM/m^2 (shell free), averaged over the entire freshwater estuary. The estimated filtration rate (normalized to 20°C) of the zebra mussel population was almost 8 $m^3 \cdot m^{-2} \cdot day^{-1}$ in October 1992. The density, biomass, and filtration rate of the population all declined from 1992 to 1994, but remained high. Because of the uneven distribution of suitable substrata in the estuary (see below), the numbers and activity of zebra mussels were likewise distributed unevenly in the estuary (Fig. 3).

Table 1. Importance of the zebra mussel population in the freshwater, tidal Hudson River.

	Zebra mussels	All zoobenthos	All heterotrophs
Biomass (g DM·m^{-2})	21	11	15
Filtration rate ($m^3 \cdot m^{-2} \cdot day^{-1}$)	6	0.1	~0.25
Respiration (g C·m^{-2}·$year^{-1}$)	105	—	570

Note: Zebra mussel data are means from 1993–1994; other data are from the preinvasion period (1986–1991). Data are from Lints et al. (1992), Howarth et al. (1992), Limburg (1993), Strayer et al. (1994), and M.L. Pace (unpublished data).

During 1993, assimilation by the zebra mussel population was estimated to be about 120–150 g C·m^{-2}·$year^{-1}$. Because this is somewhat larger than current rates of phytoplankton production in the Hudson (estimated to be about 50 g C·m^{-2}·$year^{-1}$; N. Caraco, Institute of Ecosystem Studies, personal communication), zebra mussels may have fed on bacteria and detritus as well as phytoplankton. The demands of the zebra mussel population are large compared either with summed inputs of organic carbon to the freshwater tidal Hudson (~820 g C·m^{-2}·$year^{-1}$; Howarth et al. 1992) or with total heterotrophic respiration in the system (Table 1).

Reproduction, survival, and growth

Survival and growth rates of zebra mussels in the Hudson have varied greatly among years. Although veligers were abundant throughout much of the summers of 1992–1994 (Fig. 4), year-class strength varied dramatically over this period, especially

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Fig. 3. Development of the zebra mussel population in different sections of the Hudson estuary: upper (RKM 213–248), middle (RKM 151–213), and lower (RKM 99–151), 1991–1994.

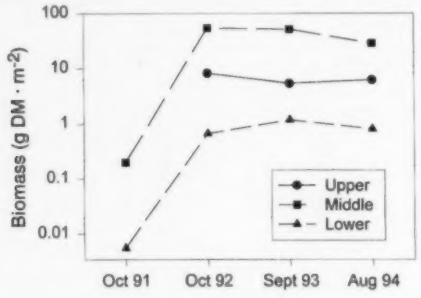
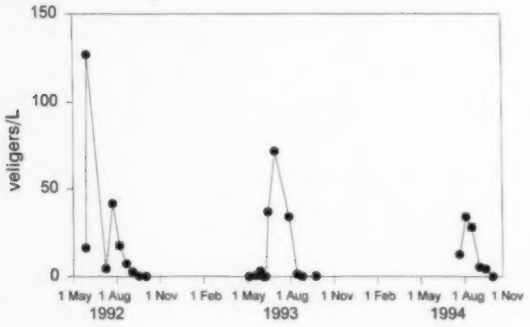


Fig. 4. Density of veligers in the Hudson River at Kingston (RKM 151), 1992–1994.



when normalized for the number of adult spawners (Fig. 5). The 1993 and 1994 year-classes were so small that they scarcely were detectable in benthic samples (Fig. 6). Growth of YOY zebra mussels likewise varied from year to year, declining sharply from the early stages of colonization (Fig. 5).

Circumstantial evidence strongly suggests that the poor growth and recruitment of YOY in 1993 and 1994 were caused by inadequate food supply. Filtration by zebra mussels reduced phytoplankton biomass in September 1992 through 1994 to 10–20% of preinvasion levels (Caraco et al. 1996). The hypothesis that an inadequate food supply limited zebra mussel growth and recruitment is consistent with the observations that both growth and recruitment were reduced after the phytoplankton crash and that recruitment was high in 1992, but postrecruitment growth was poor. (The phytoplankton crash occurred in August–September, after much settling could have occurred but before most postsettling growth.)

We doubt that environmental conditions, inadequate space for larval settlement, cannibalism of larvae by adult zebra mussels, or predation by other species could have been responsible for fluctuations in YOY recruitment and growth. In 1993–1994, important environmental variables such as temperature, freshwater flow, nutrients (N, P), and calcium were well within the ranges observed in previous years. As for local competition for space, even substrata that lacked adults during the settling season (e.g., navigation buoys, docks, boat hulls) were not heavily colonized in 1993–1994 (Fig. 5 and unpublished observations). Adult zebra mussels can consume their own larvae (MacIsaac et al. 1991), and filtration rates of zebra mussels in the Hudson were high (Table 1), so cannibalism may have been partly responsible for the poor recruitment in 1993–1994. Cannibalism cannot be the only important mechanism, though; it is hard to see how cannibalism by itself could have reduced the size as well as the number of YOY. Although predators (e.g., fish, blue crabs (*Callinectes sapidus*)) in the Hudson eat zebra mussels (Molloy et al. 1994; Limburg and Ahrend 1995; Chandler and Schmidt 1995), we do not think they were responsible for the poor growth and recruitment of zebra mussels in 1993–1994. Unless the controlling predator

Fig. 5. Numbers and growth of young-of-the-year (YOY) zebra mussels in the Hudson estuary, 1991–1994. (*a*) YOY index (i.e., average number of YOY on navigation buoys per metre circumference). Line shows means and other symbols show 95% confidence limits, as estimated by bootstrap methods. Note logarithmic scale. (*b*) YOY index normalized for the estimated number of adult spawners in the estuary. Note logarithmic scale. (*c*) Mean body mass (mg DM, shell free) of YOY (n = 166–485) taken from navigation buoys at the close of the growing season. Vertical lines show 95% confidence interval around the means, estimated by conventional statistical methods.

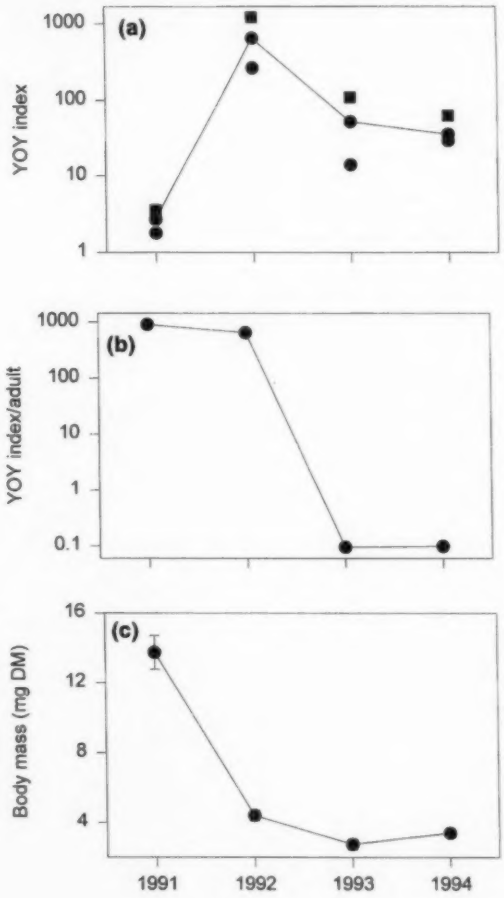


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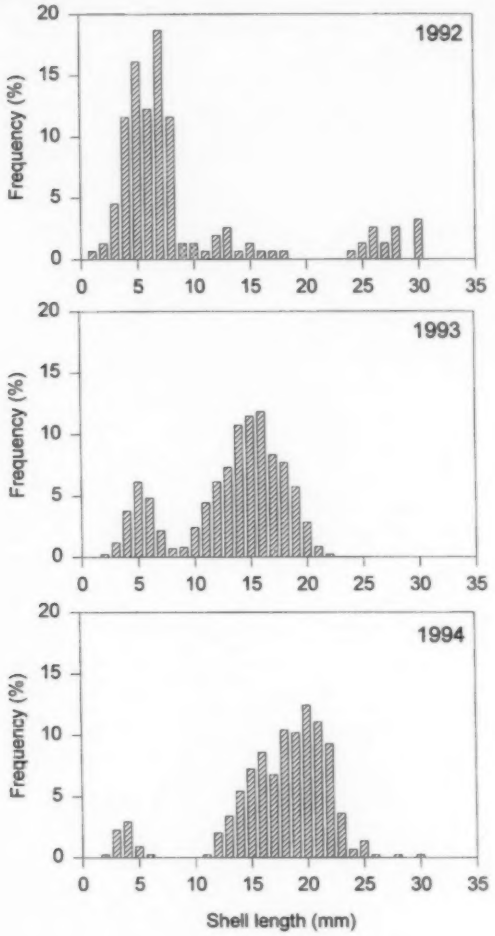
Fig. 6. Size distribution of zebra mussels in the Hudson River in September, 1992–1994.



selectively consumed the larger YOY or had a strongly seasonal activity (allowing only the tail end of the year-class to survive), it would not have caused the YOY to be both smaller and fewer in number. We do not know of any abundant predator in the Hudson that meets these requirements. Furthermore, blue crabs were very scarce in the freshwater tidal Hudson in 1993–1994 but abundant in 1992 (J. Powell, unpublished data).

Ecological distribution within the estuary

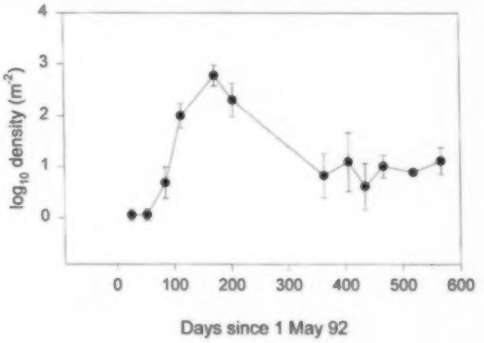
The distribution of zebra mussels in the Hudson estuary is strongly controlled by the distribution of suitable substrata (Table 2). The highest densities are found on rocks in deep (>5 m) water. Even though such deep-water rocky areas cover only 7% of the estuary, they support >95% of the zebra mussel population. Rocky areas in shallow water occasionally support modest numbers of zebra mussels but are wiped nearly clean in the winter, probably by ice and severe cold (Fig. 7). In shallow waters, zebra mussels are restricted almost entirely to the tops of rocks, in contrast to deep water, where tops, sides,

Table 2. Average densities of zebra mussels on various substrata in the Hudson River estuary, September–October 1993.

Substratum	Density (no./m^2)
Rocks in deep (>5 m) water	17 000
Soft sediments	80
Unionid clams	4.4[a]
Rocky shoreline[b]	50

[a]Equivalent to 0.5 zebra mussels per unionid.
[b]Between 0.3 m above and 0.3 m below mean low tide.

Fig. 7. Population density of zebra mussels on a rocky shoreline in the Hudson River, 1992–1993. Data are means of three elevations (see text); vertical bars show 95% confidence intervals around the means, as estimated by conventional statistical methods. Note the winter decline in densities and the absence of a 1993 year-class.



and bottoms are well colonized. Fine-grained sediments (sand, silt, and clay) are populated sparsely by zebra mussels, which live on the numerous objects (e.g., sticks, coal, stones, shells) embedded in such fine sediments. These scattered colonizable objects have not served as nuclei for large zebra mussel colonies in the Hudson, as has been observed in some other rivers and estuaries (Mordukhai-Boltovskoi 1960; Sparks 1994).

Although living unionid clams are heavily infested by zebra mussels in many lakes and rivers (see Ricciardi et al. 1995 for a review) and were predicted to support a large population of zebra mussels in the Hudson (Strayer and Smith 1993; Strayer et al. 1994), they scarcely have been colonized up to 1994. According to the model of Ricciardi et al. (1995), the average infestation in the Hudson should be 53 mussels/unionid, which would translate to 426 mussels/m^2 on unionids. We do not know why unionids are so lightly infested in the Hudson or whether this light infestation is transient or permanent.

In addition to the substrata listed in Table 2, macrophytes (especially *Vallisneria*) in the Hudson support some zebra mussels during the late summer and fall. We have not estimated the size of such plant-supported populations, which are substantial in some systems, at least seasonally (e.g., Lewandowski 1982).

General discussion

The chronology of the zebra mussel invasion of the Hudson confirms two generalizations about zebra mussel populations. First, the appearance of a disjunct population in the middle of the Hudson shows the importance of human vectors in moving

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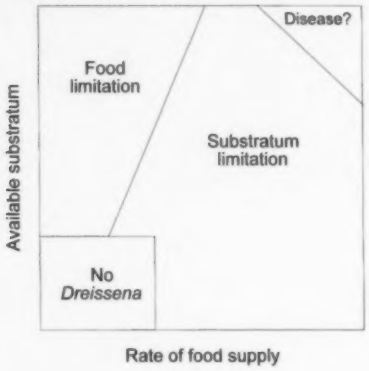
Fig. 8. Suggested domains of substratum and food limitation of zebra mussel populations.



zebra mussels across country, as has been seen elsewhere in North America and Europe. Second, population growth in the Hudson was extremely rapid, providing yet another example of the explosive potential of zebra mussel populations. Only 17 months after their first detection in the Hudson, zebra mussels constituted >70% of zoobenthic biomass and had dramatically altered the pelagic ecosystem (Caraco et al. 1996).

A third generalization, that zebra mussel populations become limited after an initial explosive phase, must of course be true, but has not been described before in detail. In the Hudson, the limitation appears to come not from external factors (predators or pathogens) or from competition among settled animals for space or food, but rather from control of larval survival and growth by competition for food with adults. This probably is not the way that most zebra mussel populations are limited, but may occur where adult impacts on phytoplankton are very severe. Such severe impacts are most likely to occur in systems where there is ample substratum for adult settlement. Furthermore, Caraco et al. (1996) argued that zebra mussels have the most severe impacts on phytoplankton biomass in systems with high concentrations of suspended inorganic particles. Thus, we might expect adult–larval competition for food to limit zebra mussel populations most frequently in turbid systems with ample rocky areas (rivers?) (Fig. 8). In systems with ample food and limited substrata (lakes?), we might expect the population to be substratum limited. In addition, the dynamics of food-limited zebra mussel populations might be erratic, as dominant year-classes develop, monopolize food resources, and then die.

The suggestion that some zebra mussel populations might be limited by food rather than substratum seems to contradict existing models for zebra mussel populations. The only effective model for predicting the population density of zebra mussels (Mellina and Rasmussen 1994) is based on substratum limitation. If the zebra mussel population in the Hudson is not limited by substratum, why does it fall on Mellina and Rasmussen's regression line? (The Hudson was one of the systems studied by these authors.) A possible resolution of this dilemma is to distinguish within-system patterns from among-system patterns of density variation. Even if food rather than substratum is limiting, density within a system should be correlated with substratum particle size. Across systems, substratum-limited populations should fall on a line, with food-limited populations in a cloud below that line. Mellina and Rasmussen's (1994) analysis showed that lakes fall on a well-defined line distinctly above a poorly defined line containing data from rivers. Perhaps this shows that many lacustrine populations are limited by substratum, while most riverine populations are limited by food, as we postulated above. If our speculative analysis is correct, not all zebra mussel populations lie in the domain of substratum-based predictive models (Fig. 8). Therefore, it will be important to determine the number, dynamics, and ecological effects of food-limited zebra mussel populations, such as the one in the Hudson.

Acknowledgements

This work was supported by grants from the Hudson River Foundation and the U.S. Army Corps of Engineers Waterways Experiment Station. We thank Chris Borg, Karyl Brewster, David Cohen, Dr. William Cook, Dean Hunter, Greg Lampman, Ron Payson, and Brian Sharp for assistance. Nina Caraco, Jon Cole, Stuart Findlay, Karin Limburg, and Peter Raymond shared their data and ideas with us. This is a contribution to the program of the Institute of Ecosystem Studies.

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Determinants of growth and body condition of lake sturgeon (*Acipenser fulvescens*)[1]

Réjean Fortin, Pierre Dumont, and Sylvie Guénette

Abstract: Determinants of variations in growth in length and body condition of lake sturgeon (*Acipenser fulvescens*) were studied on 32 lake and river systems covering most of the distribution of the species. The average of mean total lengths at ages 23–27 years and the weight of a 1000-mm specimen were chosen as growth and condition indicators. A latitudinal gradient in temperature, pH, and conductivity characterizes the eastern part of the distribution (≤80°W) whereas the western part (>80°W) shows essentially the same temperature gradient but relatively high and stable pH and conductivity values from south to north. Growth decreases with mean annual air temperature and latitude in both sectors, but it is generally faster in the west than in the east. Although mean annual air temperature was the best individual predictor of growth ($R^2 = 0.53$), a multivariate model incorporating latitude and longitude explained a higher proportion of the variation in the dependent variable ($R^2 = 0.67$). Condition decreases with latitude in the east but remains relatively stable in the west. pH was the best individual predictor of condition ($R^2 = 0.43$).

Résumé : Le déterminisme de la croissance en longueur et de la condition chez l'esturgeon jaune (*Acipenser fulvescens*) a été étudié au sein de 32 systèmes de lacs et de rivières couvrant la majeure partie de l'aire de distribution de l'espèce. La moyenne de la longueur totale moyenne aux âges 23 à 27 et le poids à 1000 mm ont été choisis comme indicateurs de la croissance et de la condition. Un gradient latitudinal de température, pH et conductivité caractérise la partie est de l'aire de distribution (≤80°W), alors que la partie ouest (>80°W) affiche essentiellement le même gradient de température, mais des pH et conductivités relativement élevés et stables du sud au nord. La croissance, généralement plus rapide dans l'ouest, diminue avec la latitude dans les deux secteurs. Bien que la température annuelle moyenne de l'air ait été le meilleur prédicteur de la croissance ($R^2 = 0.53$), un modèle multivarié incorporant la latitude et la longitude a expliqué une plus forte proportion de la variation de la croissance ($R^2 = 0.67$). La condition diminue avec la latitude dans l'est, mais demeure relativement stable dans l'ouest. Le pH a été le meilleur prédicteur de la condition ($R^2 = 0.43$).

Introduction

Early studies on the growth of lake sturgeon (*Acipenser fulvescens*) were mostly descriptive and used the slow growth, along with other characteristics of the life cycle (delayed sexual maturity, long time interval between spawnings, etc.), to document the fish's low resilience to exploitation. Growth has also been incorporated in yield per recruit type models to study the impact of exploitation on populations and to estimate production and biomass (Sandilands 1987; Dumont et al. 1987; Nowak and Jessop 1987). Several authors have compared qualitatively the growth curve of the population under study with that of reference populations from lakes Winnebago, Wisconsin (Priegel and Wirth 1975), St. Pierre, Quebec (Cuerrier 1966), St. Francis, Quebec (Cuerrier and Roussow 1951), and Nipigon, Ontario (Harkness 1923). These comparisons suggested that lake sturgeon growth decreases with latitude (Mosindy 1987). In their study of lake sturgeon biology in 14 lakes and river systems in Quebec, Fortin et al. (1992) showed that sex was a major determinant of growth and condition, females showing a tendency to become longer than males at a given age, beyond ages 25–30 years, and heavier at a given length. Moreover, growth and condition were found to differ significantly between systems and to decrease significantly with latitude. However, studies conducted in northern Ontario (Groundhog and Mattagami rivers) revealed that growth at these high latitudes can be as fast as in some populations from the southern portion of the distribution (Nowak and Jessop 1987). A synthetic study aimed at identifying determinants of growth and condition, which are often used as indicators of the general well being of fish populations, may help to establish regional standards and provide insight into factors affecting the productivity of lake sturgeon populations.

The major objective of this study was to compare growth and condition in 32 lake and river systems and (or) subsystems from a wide range of latitudes and longitudes across the distribution of the species (Quebec, Ontario, New York, Manitoba, Saskatchewan, and Wisconsin) in relation with environmental variables. Our hypothesis was that growth and condition in lake sturgeon vary with latitude, longitude, temperature, and water chemistry.

Materials and methods

The data bank used for the statistical analyses includes 18 lake and river systems and (or) subsystems from Quebec (five sections of the St. Lawrence River, Lac des Deux Montagnes, lower Ottawa River,

Received August 18, 1995. Accepted November 20, 1995.
J13045

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[1] This study is dedicated to J.P. Cuerrier and E. Magnin †, two pioneers of lake sturgeon research.

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Table 1. Mean of mean lengths at ages 23–27 years (TL_{23-27}) and weight of a 1000-mm specimen ($W_{1000\ mm}$) of lake sturgeon from 32 lakes or river sectors across the distribution of the species, and latitude (LAT), longitude (LON), mean annual air temperature (TEMP), conductivity (COND), and pH.

Study	System	$W_{1000\ mm}$ (g)	TL_{23-27} (mm)	LAT (°N)	LON (°W)	TEMP (°C)	COND (μS)	pH
	East (≤80°W)							
Fortin et al. 1992	Lake Saint-Louis	6461	1241	45	74	6.6	290	8.3
Joliff and Eckert 1971	Upper St. Lawrence River	6763	1262	45	76	7.3	314	8.5
Cuerrier and Roussow 1951	Lake Saint-Francis	5872	1138	45	74	6.3	295	8.1
Fortin et al. 1992	Lower Ottawa River	6437	989	46	77	5.7	63	7.3
Mongeau et al. 1982	Lac des Deux Montagnes	5456	1143	46	74	6.2	85	7.5
Fortin et al. 1991	Lake Saint-Pierre	6572	1200	46	73	5.8	269	8.1
Magnin 1963	St. Lawrence River (Québec City)	6181	1166	47	71	5.1	251	8.1
Fortin et al. 1991	St. Lawrence River (Gentilly)	6310	1254	47	72	4.6	246	8.1
Fortin et al. 1992	Baskatong Reservoir	6132	1177	47	76	3.2	32	7.3
Fortin et al. 1992	Témiscamingue	5413	1072	47	80	3.2	70	7.0
Fortin et al. 1992	Mégiscane W.	5056	1003	48	76	0.7	19	6.1
Fortin et al. 1992	Guéguen	5468	1118	48	77	1.5	24	6.7
Fortin et al. 1992	Mégiscane E.	5231	1023	49	76	0.7	26	6.0
Fortin et al. 1991	Harricana	5895	1078	49	79	0.1	53	7.2
Fortin et al. 1992	Bell	5420	1040	49	77	1.2	33	6.7
Fortin et al. 1992	Nottaway	5208	1040	50	77	–0.5	35	7.1
Fortin et al. 1992	Rupert	5468	1058	51	75	–1.0	25	7.2
Fortin et al. 1992	Eastmain-Opinaca	4129	659	52	77	–1.7	23	6.5
Magnin 1977	La Grande Rivière	4692	769	54	78	–3.6	15	6.5
	West (>80°W)							
Larson 1988	Lake Wisconsin	5728	1491	43	90	8.3	203	7.3
Priegel 1973	Lake Winnebago	6435	1488	44	88	1.2	335	8.1
Priegel and Wirth 1978	Upper Wisconsin Lakes	5721	1351	44	89	7.6	292	8.5
Preston, G., OMNR, personal communication	Lake Nipissing	5460	1383	46	80	4.6	78	7.4
Priegel 1973	Menominee River	5100	1321	46	88	7.6	300	7.1
Scholl 1986	Flambeau River	5305	1191	46	91	4.6	98	7.6
Nowak and Jessop 1987	Groundhog and Mattagami rivers	6515	1213	49	82	0.6	123	7.7
Mosindy 1987	Lake of the Woods – Rainy River	6119	1361	49	95	2.3	81	7.5
Harkness 1923	Lake Nipigon	5185	1076	50	89	–0.5	152	7.3
Sandilands 1987	Kenogami River	5391	848	50	84	0.17	125	7.5
Threader and Brousseau 1986	Moose River	5614	1065	51	81	–1.1	124	8.4
Wallace 1991	Saskatchewan River	5366	1015	54	98	0.3	367	8.1
Sunde 1961	Nelson River	6302	1144	55	102	–2.1	300	8.0

Note: Data are partitioned on a longitudinal basis (east vs. west). OMNR, Ontario Ministry of Natural Resources.

Baskatong Reservoir, Témiscamingue, Bell, Harricana, Mégiscane West, Mégiscane East, Guéguen, Nottaway, Rupert, Eastmain-Opinaca, and La Grande), 1 from New York (upper St. Lawrence River), 5 from Wisconsin (Lake Winnebago, lakes Poygan –Winneconne – Butte des Morts, Lake Wisconsin, upper north fork of the Flambeau River, Menominee River), 6 from Ontario (Lake Nipissing, Lake of the Woods – Rainy River, Lake Nipigon, Kenogami River, Groundhog and Mattagami rivers, Moose River), 1 from Saskatchewan (Saskatchewan River), and 1 from Manitoba (Nelson River), for a total of 32. The studies from which data were obtained were published as far back as 1923 (Harkness 1923), but most are from the last 30 years (Table 1). Harkness (1923) used otoliths to age Lake Nipigon sturgeon; all other authors used transverse sections of the marginal ray of the pectoral fin (Cuerrier 1951). Lengths and weights were generally measured on dead, fresh specimens obtained from commercial or sport fishermen or from experimental samplings by various agencies; in some cases (mark–recapture experiments), specimens were measured and weighed live.

Because of the difficulty in obtaining individual data on length, weight, sex, and age of sturgeon from all of the systems and subsystems in which these variables have been measured in the past, we chose one indicator of growth and one of body condition that can readily be calculated from the literature. The mean of the mean total lengths at ages between 23 and 27 years (TL_{23-27}, in millimetres), sexes pooled, was calculated either from observed means at ages or from values derived from growth equations (von Bertalanffy or others). Several reasons justified this choice over traditional indicators such as parameters of the von Bertalanffy equation. Although the von Bertalanffy equation was calculated by several authors, the values of the parameters cannot readily be compared from one system to another because of the great variability in the range of ages used in the estimations. On the other hand, age groups 23–27 are usually abundant in sport and commercial fishery samples, and they correspond to the range of ages when females reach sexual maturity, which is around age 25 (Cuerrier 1966; Priegel and Wirth 1971; Goyette et al. 1988). Moreover, a lake sturgeon 23–27 years old can be considered relatively young, and the precision of the age determinations should be quite reliable over this range. This age range should also be relatively free of bias because of the dome-shaped selectivity curve of commercial gill nets (Fortin et al. 1992), which tend to select fast-

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Table 2. Pearson r correlation matrix between the two dependent variables (TL_{23-27} and $W_{1000\ mm}$), and latitude (LAT), longitude (LON), mean annual air temperature (TEMP), conductivity (COND), and pH.

		TL_{23-27}	LAT	LON	TEMP	COND	pH
$W_{1000\ mm}$	C	0.59***	–0.43*	–0.03	0.49**	0.50**	0.67***
	E	0.85***	–0.82***	–0.48*	0.82***	0.72***	0.81***
	W	0.35	0.06	0.16	–0.07	0.09	0.34
TL_{23-27}	C		–0.67***	0.26	0.73***	0.50**	0.49**
	E		–0.82***	–0.50*	0.76***	0.65**	0.72***
	W		–0.73**	–0.04	0.79**	0.13	–0.13
LAT	C			0.28	–0.93***	–0.27	–0.27
	E			0.43	–0.96***	–0.67**	–0.68**
	W			0.42	–0.92***	0.07	0.23
LON	C				–0.10	0.34	0.20
	E				–0.56*	–0.69**	–0.63**
	W				–0.16	0.53	0.13
TEMP	C					0.54**	0.45**
	E					0.78***	0.79***
	W					0.24	–0.18
COND	C						0.79***
	E						0.91***
	W						0.40

Note: Statistical significances are *, P 0.05; **, P 0.01; ***, P 0.001. C, complete data set; E, east; W, west.

growing individuals in the young recruits and slow-growing individuals at the other end of the curve. Finally, in the cases where growth differences between sexes were observed in the various Quebec systems, they appeared beyond ages 25–30, so that this factor, and possible differences in sex ratio between populations, should not influence the results of the analyses.

As an indicator of body condition, we chose the weight of a specimen of 1000 mm TL (sexes pooled), calculated from the published length–weight relationships ($W_{1000\ mm}$, in grams). A reference length of 1000 mm corresponds approximately to the modal total length in the commercial catches in Quebec. The comparison of weight for a reference length is necessary because the length–weight relationship is generally not isometric in lake sturgeon (slope > 3; Fortin et al. 1992).

When length was reported as fork length (FL), it was converted to TL with the following equation:

$$TL = 1.06\ FL + 35.97, \quad R^2 = 0.98, N = 1124$$

computed for St. Lawrence River sturgeon (Fortin et al. 1992), where TL and FL are expressed in millimetres. This relationship is practically identical to the one obtained for Lake of the Woods – Rainy River sturgeon (T. Mosindy, Ontario Ministry of Natural Resources, personal communication):

$$TL = 1.06\ FL + 38.93, \quad R^2 = 0.99, N = 552$$

Covariance analyses, bivariate and multivariate correlation, and regression analyses were used to identify the determinants of variations in growth and body condition. Independent variables tested were latitude and longitude (taken from publications or estimated from maps), mean annual air temperature (estimated as the long-term annual average from the nearest reporting station; Wernstedt 1972), pH, and conductivity (usually mean values for open-water season, taken from publications or obtained from various governmental agencies). pH measurements were not available for the Kenogami River, Ontario. Therefore, pH and conductivity values for Chipman Lake, located immediately upstream of the study sector on the Kenogami River, were used in the analyses. Other variables generally used as predictors of fish production (total dissolved solids, alkalinity, indicators of primary and secondary production) could not be obtained for all of the systems or subsystems included in the data set.

Results

Mean annual air temperature showed a latitudinal gradient in the complete data set (Pearson $r = -0.93$, $P = 0.0001$, $N = 32$), while pH and conductivity were not significantly correlated with latitude (Table 2). Partitioning the data on a longitudinal basis (east vs. west of 79°W, $N = 19$ and $N = 13$, respectively; Tables 1 and 2) revealed a similar latitudinal gradient in mean annual air temperature in both sectors, no differences ($P > 0.05$) in slope or intercept being detected by a covariance analysis (Fig. 1); however, mean annual air temperatures were slightly higher at higher latitudes in the west (Fig. 1, Table 1). The two geographical sectors differed mainly by the fact that the pronounced latitudinal gradient in pH and conductivity observed in the east was not present in the west, where relatively high and variable values occurred from south to north, with no very low values at high latitudes, as in the east (Fig. 1, Table 2). Thus, the colinearity observed between temperature, pH, and conductivity in the complete data set was due to the eastern part of the distribution (Table 2).

TL_{23-27} varied between 659 mm (Eastmain–Opinaca) and 1491 mm (Lake Wisconsin), whereas $W_{1000\ mm}$ varied between 4129 g (Eastmain–Opinaca) and 6763 g (upper St. Lawrence River) (Table 1). TL_{23-27} and $W_{1000\ mm}$ were correlated in the complete data set ($r = 0.59$, $P = 0.0004$; Table 2). However, the correlation was due to the east ($r = 0.85$, $P = 0.0001$), the west showing no significant correlation between the two variables ($r = 0.35$, $P = 0.24$).

Among the five independent variables available (latitude, longitude, mean annual air temperature, pH, conductivity), mean annual air temperature, followed by latitude, showed the highest Pearson correlation with TL_{23-27} in the complete data set ($r = 0.73$ and -0.67, respectively; $P = 0.0001$; Fig. 1, Table 2). Partitioning the data increased the correlation with both independent variables (Table 2). Covariance analyses showed that the slope of the TL_{23-27} – mean annual air temperature and latitude relationships did not differ significantly

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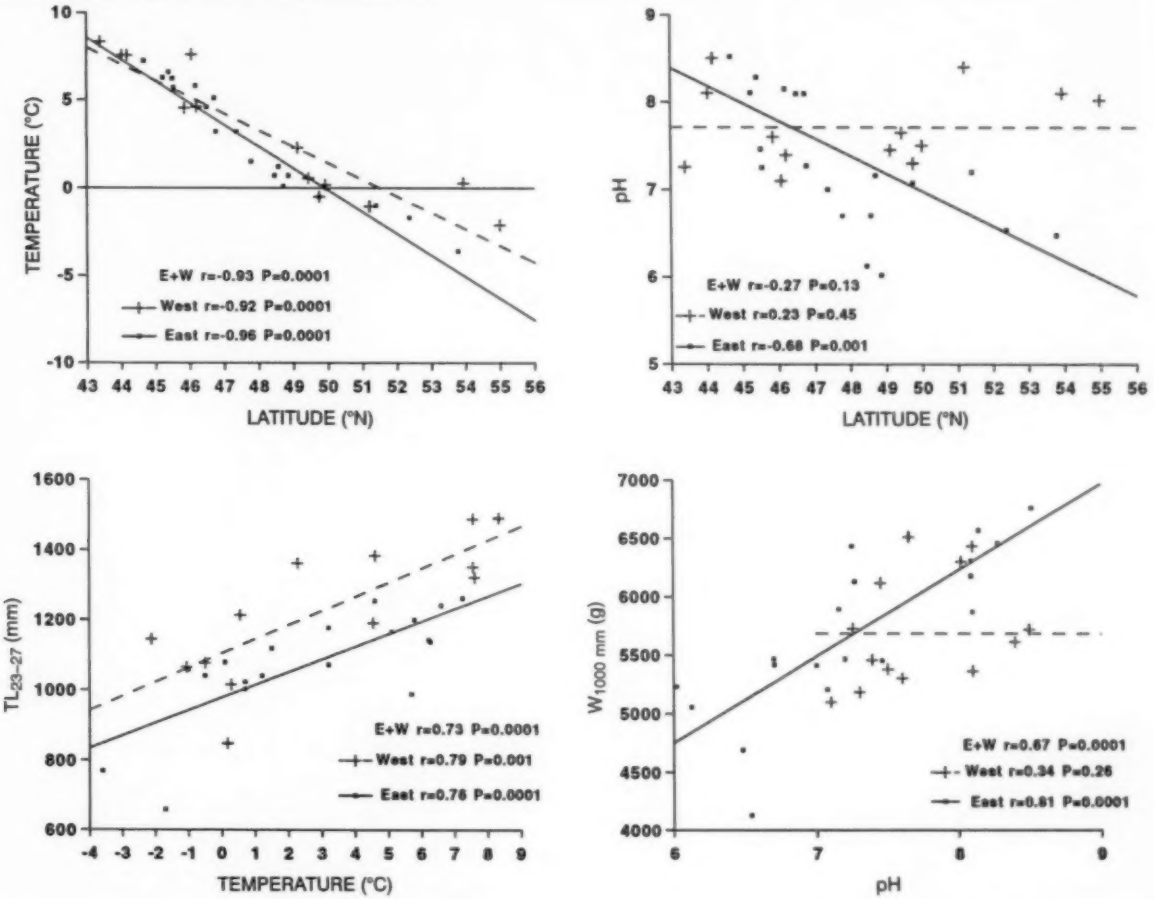
Fig. 1. Plots of mean annual air temperature and pH versus latitude, TL_{23-27} versus mean annual air temperature, and $W_{1000\ mm}$ versus pH. Pearson r correlations are given for the complete data set (E + W; $N = 32$) and for the western ($N = 13$) and eastern ($N = 19$) parts of the distribution. Lines represent regression equations for east and west. When no significant relationship was observed, a horizontal line was drawn to represent the mean of the dependent variable.



between east and west, whereas the intercepts did, TL_{23-27} being higher (ca. 150 mm) at a given temperature and latitude in the west.

The correlations observed between TL_{23-27} and pH ($r = 0.49$, $P = 0.005$) and conductivity ($r = 0.50$, $P = 0.003$) in the complete data set were due to the contribution of the east, the west showing no significant correlations with these variables (Table 2). TL_{23-27} was not correlated with longitude ($r = 0.26$, $P = 0.14$) in the complete data set; correlation between these two variables was significant in the east ($r = -0.50$, $P = 0.03$) but not in the west ($r = -0.04$, $P = 0.91$).

Because of colinearity problems in the complete data set, mean annual air temperature, although it showed the highest bivariate correlation with TL_{23-27}, was not retained in multivariate regression models when the five independent variables were included in the analysis. The best model, accounting for colinearity, predicted TL_{23-27} from latitude (LAT) and longitude (LON):

$$TL_{23-27} = 2569.6 - 49.1\ LAT + 11.4\ LON,$$
$$R^2 = 0.67,\ P = 0.0001,\ N = 32$$

in which the three coefficients were significant ($P < 0.001$). pH was not included in this model, but it nearly reached the 0.05 significance level used as a cut-off point ($P = 0.078$). When only mean annual air temperature, conductivity, and pH were included in the analysis, neither pH nor conductivity explained a significant proportion of the residual variation in TL_{23-27} once mean annual air temperature (TEMP) was included in the model:

$$TL_{23-27} = 1025.2 + 39.1\ TEMP,$$
$$R^2 = 0.53,\ P = 0.0001,\ N = 32$$

Both coefficients in the model were significant ($P < 0.001$).

Some of the data points included in the analyses were regional outliers. For example, the TL_{23-27} value for the Eastmain–Opinaca River (659 mm; Table 1) was well below the growth – mean annual air temperature linear relationship determined for the eastern part of the distribution (Fig. 1). The same was observed for the Kenogami River, where the TL_{23-27} value (848 mm; Table 1) was also below the linear relationship determined for the western part of the distribution

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(Fig. 1). Differences in age interpretation may be responsible for these discrepancies. Nevertheless, when the regression analyses were performed without these two data points, mean annual air temperature remained the best individual predictor of TL_{23-27} ($R^2 = 0.52$, $P = 0.0001$, $N = 30$). The best multivariate model still incorporated latitude and longitude ($R^2 = 0.69$, $P = 0.0001$, $N = 30$). Thus, the regional outliers seemed to have little or no influence on the general relationships derived from the data set.

pH showed the highest correlation with $W_{1000\ mm}$ in the complete data set (Fig. 1, Table 2; Pearson $r = 0.67$, $P = 0.0001$), followed by conductivity ($r = 0.50$, $P = 0.003$), temperature ($r = 0.49$, $P = 0.005$), and latitude ($r = -0.43$, $P = 0.01$). These correlations were due to the contribution of the east ($r = 0.81$, $P = 0.0001$; $r = 0.72$, $P = 0.0005$; $r = 0.82$, $P = 0.0001$; and $r = -0.82$, $P = 0.0001$, respectively; Table 2), the west showing no significant correlations with these variables.

Multiple regression analyses of $W_{1000\ mm}$ on the five independent variables in the complete data set, or on mean annual air temperature, conductivity, and pH, showed that once pH was included in the model, no other variable explained a significant proportion of the residual variation, although latitude nearly reached the 0.05 significance level ($P = 0.06$):

$$W_{1000\ mm} = 1239.3 + 598.3\ \text{pH}, \quad R^2 = 0.45,\ P = 0.0001,\ N = 32$$

in which the slope differed from 0 ($P = 0.0001$), but not the intercept ($P > 0.05$).

Discussion

Some of the data used in the present study were collected at least 70 years ago and the question of temporal change in growth and condition of lake sturgeon becomes pertinent. For three systems (St. Lawrence River, Ottawa River, and Nottaway), growth variations over time can be inferred from qualitative comparisons with data from the late 1940s available in Cuerrier (1966; Lake Saint-Pierre), Dubreuil and Cuerrier (1950; Ottawa River at the level of the Fitzroy–Quyon dam), and Magnin (1966; Nottaway system). Growth did not show major differences over time in the three systems. Mean lengths at ages younger than 40 years were relatively similar, except for the Ottawa River, where young specimens seemed shorter in the late 1940s, and sturgeon aged 50 years and older seemed longer. However, mean lengths at older ages are often derived from very small samples (1 or 2 specimens).

The Fulton condition index remained constant through time in three of four systems in Quebec. In the Nottaway system, the general mean of the Fulton index calculated from Magnin's (1966) data was 0.53, compared with the 0.52 value observed in 1988 and 1990 samples (Fortin et al. 1992). In Lac des Deux Montagnes, the mean value of K for the 1964–1978 period was 0.51 (Mongeau et al. 1982), which is identical to the value observed in 1985 and 1988 samples (Fortin et al. 1992). In 1947 and 1948, the condition index of juveniles was 0.61 in Lake St. Pierre, while that of mature females was 0.70 (estimated from data presented by Cuerrier 1966); during the 1980s, the general mean of K (commercial size fish) was 0.65 (Fortin et al. 1992). Condition changed markedly in the Ottawa River from a mean value of 0.55 (Dubreuil and Cuerrier 1950) to 0.68 in the 1980s (Fortin et al. 1992), a 24% increase. The drastic decline in abundance of lake sturgeon in the lower Ottawa River between the 1940s and the 1980s owing to commercial exploitation prompted managers to close the commercial fishery in 1992.

Our results indicate that growth in length and body condition in lake sturgeon behave somewhat differently in the eastern and western parts of the distribution. Growth rate decreased markedly with decreasing mean annual air temperatures and increasing latitudes, temperature being the best individual predictor in the complete data set and in the western part of the distribution. However, at a given temperature and latitude, fish from the west showed greater mean lengths than those from the east (difference of approximately 150 mm). This situation is integrated by the multivariate model incorporating latitude and longitude, which explains 67% of the variation in TL_{23-27}, as compared with 53% for the model incorporating only mean annual air temperature. Higher pH and conductivity values at intermediate and high latitudes in the west were possibly responsible for the higher TL_{23-27} values observed in this part of the distribution, as suggested by the fact that pH and conductivity were significantly correlated with TL_{23-27} in the complete data set and in the eastern part of the distribution and that the contribution of pH to the explanation of growth variations in the multivariate analyses nearly reached the 0.05 significance level. Our results are thus consistent with those of Nowak and Jessop (1987), who mentioned that growth in northern Ontario rivers such as the Groundhog and Mattagami can be as fast as in the southern part of the distribution. However, pH and conductivity are probably not the only factors responsible for the differences in growth rate between the eastern and western parts of the distribution. The fact that TL_{23-27} values in the southern part of the distribution were also higher in the west than in the east, whereas mean annual air temperatures, pH, and conductivity were equivalent, needs to be investigated further. Longitude in the multivariate model is probably a surrogate for other variables influencing growth, which are yet to be identified.

Temperature, edaphic variables (total dissolved solids, conductivity, alkalinity, etc.), and pH have been included in several models as predictors of fish production (Moyle 1956; Ryder 1965; Ryder et al. 1974; Matuszek 1978; Schlesinger and Regier 1982; Downing and Plante 1993). Fish production is generally higher in more mineralized, buffered, alkaline waters and at higher mean annual temperatures. Invertebrate production in lakes is also positively correlated with temperature (Plante and Downing 1989). Growth–temperature relationships have been determined for several fish species in laboratory experiments (Brett 1979). Growth in the wild has been shown to decrease with latitude in several species, whereas at a given latitude, it will show interannual variations in relation with length of the growing season and mean summer temperatures (see Colby et al. 1979 for walleye, *Stizostedion vitreum vitreum*). Colby et al. (1979) also inferred, from Carlander's (1948) data on some Iowa lakes, that walleye growth rates are directly related to the productivity of a body of water. Our results suggest that lake sturgeon growth follows this general pattern: it decreases with latitude and mean annual temperatures but is faster in more alkaline and conductive (and probably more productive) waters.

An unexpected result is the fact that lake sturgeon can over-

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come the latitudinal temperature gradient and maintain relatively stable body condition in the more alkaline and conductive waters of the western part of the distribution, whereas a latitudinal gradient in temperature, pH, and conductivity in the eastern part causes a general decrease in this variable. Different trends in condition in the two geographical sectors strongly suggest that pH and conductivity are major determinants of this variable across the distribution of the species. Although the multiple regression analysis identified pH as the best predictor of body condition, conductivity (and hence total dissolved solids) should also be considered as major determinants. Conductivity is colinear to pH in the data set, and bivariate correlation coefficients with condition (particularly in the eastern part of the distribution) and the partial R^2 values of the two variables in multivariate analyses are relatively similar.

Doyon et al. (1988) interpretated the increase in body condition of northern pike (*Esox lucius*) with latitude (while growth decreases; see Vallières and Fortin 1988) as a possible adaptation for shorter growing seasons in the north. The situation is somewhat different in lake sturgeon. High condition values, equivalent to those observed in the south, were observed in the northwestern part of the distribution but not in the northeastern part. From a management standpoint, it would be interesting to verify whether the higher body condition in the northwestern part of the distribution is associated with shorter intervals between spawnings and (or) possibly higher fecundity than in the northeastern part.

Acknowledgements

We acknowledge all collaborators who provided individual data on lake sturgeon and (or) environmental data on the various lakes and rivers for which growth and condition data were available: Martin Léveillé, Yves Mailhot, Martin Rousseau, Jean-René Mongeau, Henri Fournier, Alain Fort, and Daniel Nadeau of the ministère de l'Environnement et de la Faune du Québec; Fritz Axelsen of the ministère des Pêcheries et de l'Alimentation du Québec; René Dion and Michel Simoneau of the Cree Grand Counsel; Dominique Roy of the Société d'énergie de la Baie James; Marc Gendron of Environment Illimité; Al Sandilands of Ecological Services for Planning Ltd.; Tom Mosindy, Jim Rusak, A.M. Nowak, C.S. Jessop, Evan Armstrong, and Gary Preston of the Ontario Ministry of Natural Resources; Tim Larson, Ron Bruch, and Jeff Roth of the Wisconsin Department of Natural Resources; Steve Lapan and Al Schivione of the New York State Department of Environmental Conservation; Robert Wallace of the Saskatchewan Department of Environment and Resource Management; and Bing Chu of the Monitoring Operations Division, Prairie and Northern Region, Environment Canada. This study was funded by the ministère de l'Environnement et de la Faune du Québec.

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A reevaluation of the Red Queen model for the maintenance of sex in a clonal–sexual fish complex (Poeciliidae: *Poeciliopsis*)

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Abstract: The validity of the assumptions of the Red Queen model for the maintenance of sexual reproduction was reassessed using life-history data collected from clonal and sexual fish in the genus *Poeciliopsis*. A previous study using these strains (one sexual, two clonal) indicated that sex might be maintained by coevolutionary processes outlined in the Red Queen model. However, the previous study did not test the assumption that parasitism negatively affects the host's fitness. The current study was undertaken to address this issue, as well as to reevaluate the assumptions of the Red Queen model in this complex. Data on size, fecundity, and parasitic intensity were collected from fish from seven Mexican pools. Parasitic intensity was highest for one clone, but intensity was not correlated with clonal frequency, as assumed by the Red Queen model. Variance in parasitic intensity was not reduced in the clonal strains relative to the sexual strain, and intensity was not correlated with fecundity. These data, combined with aspects of the parasite's biology, indicate that the Red Queen model is unlikely to maintain sex in this clonal–sexual complex.

Résumé : La validité des suppositions relatives au modèle Red Queen pour l'entretien de la reproduction sexuelle a été réévaluée à l'aide des données historiques recueillies à partir de poissons d'origines clonale et sexuelle du genre *Poeciliopsis*. Une étude antérieure, fondée sur ces souches (une sexuelle, deux clonales), a montré que la reproduction sexuelle pourrait être entretenue par les processus de coévolution décrits dans le modèle Red Queen. Cependant, l'étude précédente n'a pas vérifié l'hypothèse voulant que le parasitisme agissait négativement sur la bonne forme de l'hôte. L'étude actuelle a été entreprise pour examiner cette question et pour réévaluer les suppositions du modèle Red Queen relatives à ce complexe. Les données sur la taille, la fécondité et l'intensité parasitaire ont été recueillies sur les poissons provenant de sept étangs mexicains. L'intensité parasitaire était la plus élevée pour l'un des clones, mais il n'y avait pas de corrélation entre l'intensité et la fréquence clonale, comme le suppose le modèle Red Queen. La variance dans l'intensité parasitaire ne se trouvait pas réduite dans les souches clonales, comparativement aux souches sexuelles, et il n'y avait pas corrélation entre l'intensité et la fécondité. Ces données, combinées à certains aspects de la biologie des parasites, montrent qu'il est peu probable que le modèle Red Queen entretienne la reproduction sexuelle dans ce complexe clonal–sexuel.
[Traduit par la Rédaction]

Introduction

In the early 1970s, Maynard Smith (1971*a*, 1971*b*) and Williams (1975) concluded that sexual reproduction has an immediate twofold numerical cost relative to asexual reproduction. This twofold cost of sex was difficult to accept considering the prevalence of sexual reproduction among higher plants and animals (Levins 1942; Bell 1982). Since then, numerous models have been formulated proposing short-term advantages of sex that might offset this cost. Hypothesized benefits of sexual reproduction range from strictly genetic (Bernstein et al. 1981, 1984, 1985; Kondrashov 1982, 1984; Kirkpatrick and Jenkins 1989) to strictly ecological (Williams and Mitton 1973; Ghiselin 1974; Hamilton 1975, 1980, 1990; Williams 1975; Vrijenhoek 1979, 1984; Anderson and May 1982; Bell 1982, 1985; Case and Taper 1986; Weeks 1993).

One of the ecological models, the Red Queen (RQ) model, ascribes a benefit to producing variable progeny that can potentially offset this twofold cost of sex in a biotically variable environment (Hamilton 1975, 1980, 1990; Levin 1975; Glesener and Tilman 1978; Jaenike 1978; Bremmermann 1980, 1985; Anderson and May 1982; Bell 1982). Sexual reproduction is predicted to allow offspring to adapt to a changing biotic environment, in which antagonists (e.g., predators and parasites) target the most common phenotypes of a population, causing frequency-dependent selection for uncommon phenotypes (Hamilton 1980; Hutson and Law 1981). This coevolution of predator and prey or parasite and host causes the offspring's environment to be predictably different from that of the parent's, which selects for genetic rearrangement of the parental genes across generations (Bell 1982). Thus, both the target population and its associated antagonists are caught in a continual cycle of genetic rearrangement, with the target species shuffling its genes to assure moving targets for predators and parasites, and the antagonists also reshuffling to genetically track their prey or host populations (Levin 1975; Jaenike 1978; Hamilton 1980, 1990; Hutson and Law 1981; Bell 1982).

Clonal and sexual fish in the genus *Poeciliopsis* have been used successfully to address many of the ecological models for the maintenance of sex. Several studies of a related ecological model, the frozen niche variation (FNV) model (Vrijenhoek 1979, 1984; Weeks 1993), have indicated that clonal and sexual strains coexist because of phenomena associated with re-

Received July 7, 1995. Accepted November 20, 1995.
J12986

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Table 1. Estimated fish populations collected from nine pools.

Pool	Estimated total number	Density (no./L)	Number collected				Position[a]
			P. monacha	MML/I	MML/II	ML/VII	
1	203	0.15	23	18	0	0	1 (near log pool)
2	390	1.14	40	8	2	0	1 (near log pool)
3	50	0.16	16	12	8	0	2 (near sandal pool)
4	210	0.39	40	10	0	0	2 (near sandal pool)
5	269	0.35	32	10	8	0	2 (near sandal pool)
6	200	0.10	47	2	1	0	3 (below sandal pool)
7	69	0.13	25	9	18	0	4 (200 m above bottom)
8	136	0.08	41	4	0	5	5 (bottom of Platanos)
9	75	0.07	42	8	2	0	5 (bottom of Platanos)

[a]Positions are outlined in Lively et al.

source partitioning. The FNV model assumes that the production of offspring capable of using different resources is adaptive in a heterogeneous environment (Vrijenhoek 1979, 1984; see also Bell 1982). Sexual populations are assumed to have a broader niche than a monoclonal population, which should reduce among-individual competition and increase overall productivity (Ghiselin 1974; Vrijenhoek 1979, 1984; Bell 1982, 1985; Case and Taper 1986; Weeks 1993). Clones should outcompete sexual individuals for the narrow range of resources to which the clones are best adapted, because of their twofold reproductive advantage, but sexual individuals are predicted to persist because their wider niche allows them use of resources unavailable to individual clones (Vrijenhoek 1984; Weeks 1993). Vrijenhoek (1979) has shown that sexual individuals constitute a smaller proportion of *Poeciliopsis* populations in streams where clonal diversity is high than they do in monoclonal streams. This sexual replacement is consistent with the notion that the clones are usurping a higher proportion of the niche space in populations where their combined resource breadth is largest (Vrijenhoek 1979). Schenck and Vrijenhoek (1986, 1989) found both spatial and dietary differences among sexual and clonal populations of two unisexual biotypes of *Poeciliopsis* that might facilitate coexistence in their native streams. Similarly, Weeks et al. (1992) described differences in feeding behavior between two naturally cooccurring hemiclones and their sexual progenitors, which corresponded with natural differences in the diets of field-caught fish, which may also help explain clonal and sexual coexistence. These studies provide strong evidence for genetically determined, ecologically relevant, phenotypic differences among clonal lineages and between clones and sexual individuals and have therefore verified many of the assumptions and predictions of the FNV model.

Recently, the *Poeciliopsis* system was also used to confirm assumptions of the RQ model. Lively et al. (1990) showed that clones in a three-pool system had higher parasitic intensity than similarly sized, outcrossed sexual individuals. Also, Lively et al. (1990) found that the most common clone was the most heavily parasitized, although the most common type differed among pools. These results suggest a tight tracking of the most common genotype by the parasite over a short geographic range, which would be a strong verification of the assumptions of the RQ model.

The results of the Lively et al. (1990) study are intriguing because they suggest that the maintenance of sexual reproduction in the *Poeciliopsis* system may be due, at least in part, to processes associated with the RQ model. However, that study did not establish a fitness effect of the parasites on the fish, which is necessary for operation of the RQ. In the current experiment, I sought to examine the relationship of parasitic intensity to fitness in these fish by collecting life-history data (size and fecundity) from field-collected fish. I was also able to extend the work of Lively et al. (1990) by using seven separate pools (as compared with three) to test the RQ model's assumption of higher parasitic intensity in clones relative to sexual populations. By combining the fecundity and parasitic intensity data, I was able to test for the expected negative effects of parasitism on one aspect of fitness (fecundity), which is required for the maintenance of sex under a coevolutionary scenario, as outlined by the RQ model.

Materials and methods

Fish strains and collection locations

Three *Poeciliopsis* strains were analyzed in this experiment: an outcrossed sexual strain (*Poeciliopsis monacha*) and two associated unisexual, triploid strains (*Poeciliopsis 2-monacha-lucida*: MML/I and MML/II; Vrijenhoek et al. 1977). The triploid *Poeciliopsis* strains reproduce gynogenetically, a strictly clonal mode of reproduction whereby the entire triploid genome is faithfully replicated among generations (Schultz 1967). Although sperm from a sexual species is required to activate embryogenesis in the triploid ova, paternal genes make no contribution to the genotype of the offspring (Schultz 1967; Cimino 1972; Vrijenhoek 1972). For further information on this reproductive complex, see Schultz (1969).

Five individuals of the hybridogenetic clone *P. monacha-lucida* (ML/VII; Vrijenhoek et al. 1978) were collected from one of the nine pools (Table 1). However, data from a single pool did not allow a statistical comparison; therefore, data from these five fish were not included in the figures or tables that follow.

Fish were collected from pools in the Arroyo de los Platanos region of the Rio Fuerte, Sonora, Mexico (26°30′N, 108°30′W; see Moore and Eisenbrey 1979 for further details of the region), during May 1990. During this time of year, the stream dries down to small, isolated pools that are interconnected by trickles of water. This isolation results in a number of pools that are essentially closed systems, with only a slight amount of migration among pools (Moore and Eisenbrey 1979). Nine of the most isolated of these pools were chosen to maximize the likelihood that the measured biotic and abiotic factors of each pool well reflected the major influences on recent growth and development of the collected fish.

The measures taken from each pool included relative position along the stream, dimensions of the pool (width, length, and depth), and total number of fish per pool. Most pools were fairly rectangular,

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Table 2. Analysis of covariance for infections per strain.

Source	df	Mean square	*F*	*P*
Pool 1				
Size	1	24.221	39.70	0.0001
Strain	1	3.257	5.34	0.0254
Size × strain	1	5.113	8.38	0.0058
Pool 2				
Size	1	0.744	0.90	0.3487
Strain	1	6.335	7.64	0.0082
Pool 3				
Size	1	25.660	42.75	0.0001
Strain	2	1.137	1.89	0.1669
Pool 4				
Size	1	48.160	56.21	0.0001
Strain	1	3.202	3.74	0.0594
Pool 5				
Size	1	46.797	49.14	0.0001
Strain	2	3.081	3.24	0.0484
Pool 7				
Size	1	3.290	6.54	0.0138
Strain	2	0.131	0.26	0.7716
Pool 9				
Size	1	0.490	0.35	0.5568
Strain	1	6.025	4.31	0.0434

Note: Pool 1 had a significant size × strain interaction, and thus this term was included in the model. All other pools had no significant size × strain interaction and thus had size × strain dropped from the model.

with sloping bottoms. Therefore, pool volume was estimated using the dimensions above and assuming the pools were longitudinally divided half cylinders. Total number per pool was estimated using a catch per unit effort model (Ricker 1958). Pools were repeatedly sampled, using a hand-held seine, until either no fish were caught, or the number was reduced to the point at which only a few fish were caught in each haul (usually five to seven seine hauls). Fish density was calculated by dividing the estimated population size by pool volume.

A sample of 50 females (when available) was taken from each pool. Tissue samples from each female were used for starch-gel electrophoresis to determine sexual type and clonal identity. Life-history characters measured were standard length and number of eggs and embryos per female. Also, the number of parasitic cysts was determined for each female (see Lively et al. 1990). The parasite is a trematode larva (*Uvulifer* sp.) that causes spherical, black cysts in the epidermis of these fish, which are easily counted under a dissecting microscope at low magnification (Lively et al. 1990).

Statistical analysis

All statistical analyses were performed using the SAS statistical package (SAS Institute Inc. 1985). In any particular pool, a clonal strain was not included in these analyses if there were less than 5 individuals in the collection of 50 fish (Table 1). Parasitic infection was analyzed using analyses of covariance, as outlined in Lively et al. (1990). The numbers of parasitic cysts per fish were compared among strains in each pool. Only one of seven comparisons indicated that the slopes of the lines relating number of cysts to body size were significantly different among strains. In this one case, analysis was discontinued at this point (see Lively et al. 1990). In the other six cases, in which the slopes were not significantly different, an analysis of covariance was performed comparing the number of cysts per unit body size among the strains within each pool.

Variance ratios were employed to test the prediction that sexual populations have a greater among-individual difference in parasitic load than clones. Variation in parasitic intensity per strain was estimated by computing the variance of the residuals of cyst number regressed on standard length (see Lively et al. 1990), both within each pool as well as combined across all pools. For the across-pool comparison, the residuals were computed from a model including standard length, pool, and the standard length by pool interaction to account for possible differences in parasitic intensity among pools. Comparisons between strains were made using the ratios of the sexual to the clonal variance and comparing these ratios with standard *F* tables using $n_s - 2$ degrees of freedom for the numerator and $n_c - 2$ degrees of freedom for the denominator, where n_s is the sample size of the sexual populations and n_c is the sample size of the clones (Sokal and Rohlf 1981).

To determine whether increased parasitic intensity was associated with reduced fecundity, size-corrected fecundity was regressed on number of cysts per individual. Size-corrected fecundity was calculated by using the residuals of fecundity regressed on standard length and adding the average fecundity to each residual score. These comparisons were made within each strain, combining individuals across all nine pools.

Results

Estimated population sizes ranged from 50 to almost 400 individuals per pool (Table 1). Because the seines used to sample these nine pools had mesh sizes small enough to sample males but too large to collect the smaller juveniles, these estimates reflect adult numbers, but underestimate the number of juveniles. Fish density ranged over an order of magnitude, from less than 0.1 to 1.1 fish/L of water (Table 1). Quantification of clonal and sexual frequencies revealed that clonal versus sexual abundance ranged from a majority of unisexual individuals (56% in pool 3) to mainly sexual individuals (94% in pool 6; Table 1).

Differences among strains in parasitic intensity were determined using a two-step analysis of covariance (see above). The relationship between intensity and size differed in only one of the seven pools (pool 1; Table 2) indicating that, in this pool, strain MML/I had a greater increase in parasitic intensity with increased body size than *P. monacha* (Fig. 1). In the remaining six pools the rate of increase in parasitic intensity with increased size did not differ among strains (i.e., slopes of parasitic intensity on body size were homogeneous). In these remaining six pools, MML/I had significantly higher size-corrected parasitic intensity than either MML/II or *P. monacha* (Table 2, Fig. 1). In the only pool where MML/II was the most abundant clone (pool 7), there was no indication that it was more heavily parasitized than MML/I (Fig. 1). In fact, in all seven pools MML/I showed evidence of being the most parasitized of all three strains, regardless of its frequency or the frequency of MML/II (Fig. 1).

Among-individual variation in susceptibility should be highest in the sexual populations, assuming a tight tracking of host susceptibility genotypes by the parasitic population. In only 3 of 10 comparisons was the variance in parasitic infection greater in the sexual than the clonal population, and none of these differences were significant (Table 3). Since within-pool sample sizes were only moderate, no single within-pool comparison of variance had high statistical power. However, sample sizes were large enough to detect a trend of a higher variance in infection rate for sexuals if one existed. Also, when results were combined across pools, increasing the statistical power to detect differences, there was no evidence of in-

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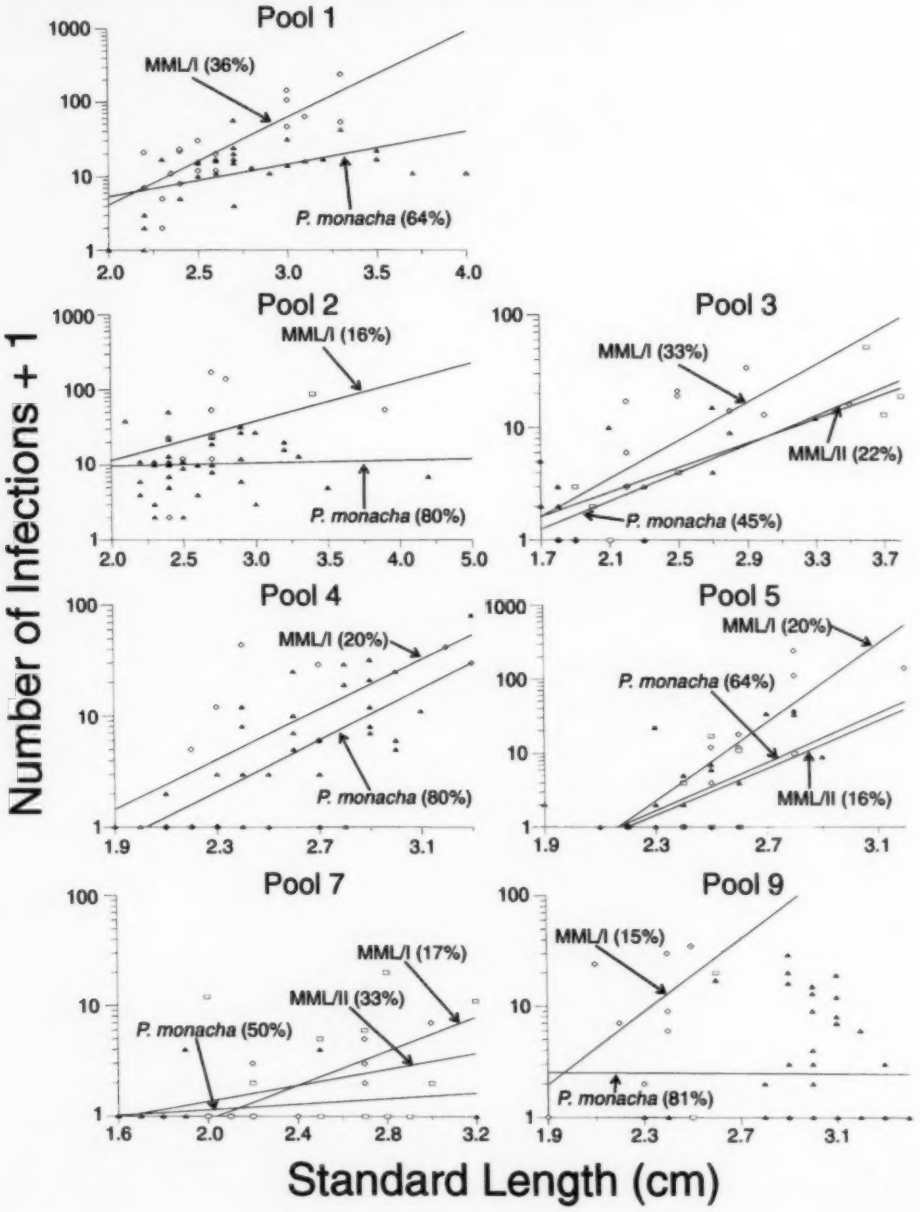
Fig. 1. Plots of parasitic intensity versus standard length for seven of the nine pools with coexisting clones and sexual individuals. Regression lines and strain frequencies (in parentheses) are shown for strains having more than five individuals per pool. Note that the axes differ among pools. Symbols are as follows: open diamonds, MML/I; open squares, MML/II; solid triangles, *P. monacha*.



creased variability of parasitic intensity for the sexual individuals relative to the clones.

Size-corrected fecundity was regressed on number of parasitic cysts per individual within strains and across pools to examine whether level of parasitism was associated with reduced fecundity (Table 4). All three strains had a negative association of size-corrected fecundity with parasitic intensity, but in no case was this association significant (Table 4). In fact, in all three strains the number of cysts per individual only accounted for between 0.4 and 3% of the variation in size-corrected fecundity (Table 4, Fig. 2).

Discussion

The RQ model assumes that biological antagonists (e.g.,

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Table 3. Comparisons of variance in parasitic intensity among sexual and clonal strains.

Comparison		Numerator	Denominator	*F*
Pool 1	Pm/C1	0.6465 (30)	0.4719 (16)	1.370
Pool 2	Pm/C1	0.5859 (38)	1.8315 (6)	0.320
Pool 3	Pm/C1	0.5301 (14)	0.7380 (10)	0.718
	Pm/C2	0.5301 (14)	0.3360 (6)	1.578
Pool 4	Pm/C1	0.7489 (36)	1.2868 (8)	0.582
Pool 5	Pm/C1	0.7935 (30)	0.9073 (8)	0.875
	Pm/C2	0.7935 (30)	1.1720 (6)	0.677
Pool 7	Pm/C1	0.1373 (23)	0.2067 (7)	0.664
	Pm/C2	0.1373 (23)	1.0066 (16)	0.136
Pool 9	Pm/C1	1.3347 (40)	1.0808 (6)	1.235
Overall	Pm/C1	0.720 (211)	0.783 (61)	0.920
	Pm/C2	0.720 (211)	0.838 (28)	0.859

Note: The sexual strain (numerator) is always compared with one or both of the clonal strains (denominator) as shown in the Comparison column. Strain designations are as follows: Pm, *P. monacha*; C1, MML/I; and C2, MML/II. Degrees of freedom for the *F* tests are shown in parentheses.

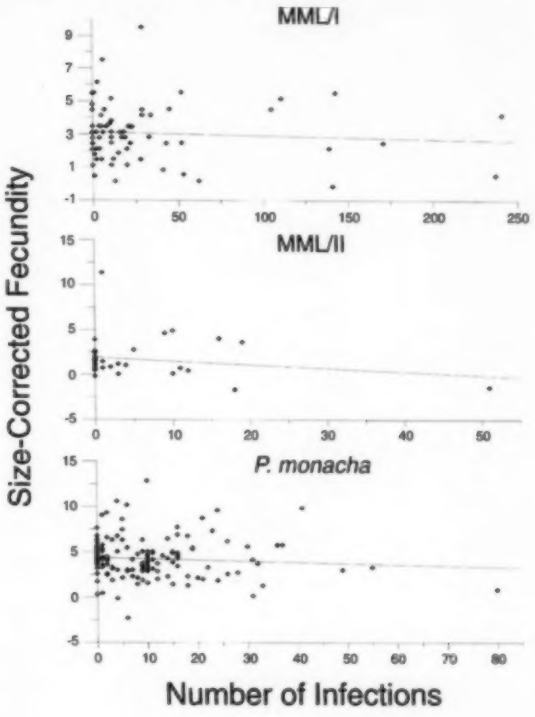
Table 4. Regression of size-corrected fecundity on level of parasitism, combining individuals across pools.

Strain	*N*	Slope	R^2	*F*	*P*
MML/I	75	–0.002	0.0036	0.266	0.6077
MML/II	34	–0.041	0.0314	1.038	0.3158
P. monacha	310	–0.021	0.0075	2.332	0.1278

predators and parasites) selectively target the most common genotype in a population, thereby disproportionately reducing that genotype's fitness relative to rarer genotypes (Hamilton 1980, 1990; Hutson and Law 1981). Such an interaction would select for the ability of prey and hosts to rearrange their genotypes to produce some number of rare genotypes that enjoy higher fitness (Hamilton 1980, 1990; Anderson and May 1982; Bell 1982). This rearrangement also selects for concomitant genotypic rearrangement in the predators and parasites, setting up the conditions for coevolution (Hamilton 1980, 1990). Three testable assumptions are important to the RQ model. First, the assumption that parasites track the most common genotype can be easily tested in clonal–sexual systems. Assuming the sexual population is sufficiently outcrossed and thus has a large number of susceptibility types, the most common clone should provide the most common susceptibility type in a population and therefore should be the most heavily parasitized. Second, a correlated assumption is that the sexual population should include a number of susceptibility types, whereas clones should be either largely susceptible or resistant. Therefore, among-individual variation in parasitism should always be highest in outcrossed sexual populations. The third assumption is that the heavily parasitized individuals must have reduced fitness relative to less parasitized counterparts. In multiclonal populations, these three processes should combine to produce fluctuating clonal dominance as rarer clones enjoy a fitness advantage, increase in frequency, and then are attacked as the new most common genotype (Lively 1993). The singling out of these individuals decreases their prevalence because they have reduced fitness concomitant with the increased parasitism.

The first two of these three assumptions were addressed by

Fig. 2. Plots of size-corrected fecundity versus number of parasitic infections, combining fish across pools. Note that the axes differ among strains.



Lively et al. (1990) using fish from the same reproductive complex and from the same stream as in the current study. In their study, three pools were sampled, two being sampled in 2 different years. Two of the three pools were monoclonal, having only MML/I and *P. monacha*. In these pools, MML/I was more heavily parasitized per unit body length than *P. monacha*, and the variance in infection intensity was highest among *P. monacha* individuals, when the *P. monacha* populations were outcrossed. Both of these observations are consistent with the RQ model. In 1 of the 2 years, the *P. monacha* population was highly inbred. During this year, *P. monacha* was the most heavily parasitized of the two strains and had a lower variance in parasitic intensity than MML/I (Lively et al. 1990). Because the sexual individuals constituted only 43% of the population during this year and thus could not have been the most common susceptibility type even if the sexuals were monomorphic at all susceptibility loci, these latter results could only be consistent with the RQ model if one assumes that the sexual individuals' overall resistance to infection was reduced by inbreeding.

The strongest evidence that the parasite tracks the most common genotype in this reproductive complex was revealed in the third pool measured by Lively et al. (1990). In this pool, MML/II was the most common clone, and it had significantly more parasites per unit length than either MML/I or *P. monacha*. There was no difference in the variance of parasitism intensity between any of the three strains. The observed rever-

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sal of the pattern of parasitism such that the most common clone was always the most heavily parasitized across a distance of no more than several hundred metres suggests an extremely tight coevolution between parasite and host in this system. This observation of greater parasitism of MML/II in this one pool is the strongest case for validation of the assumptions of the RQ model in this reproductive complex.

In the current experiment, MML/I was clearly more heavily parasitized than *P. monacha* in all pools where the two strains coexist. These data are consistent with the findings of Lively et al. (1990). However, there is no evidence that *P. monacha* had a higher variance for infection intensity in any pool, even when averaging across all pools. Moreover, in the one pool where MML/II was most common (pool 7), there was no evidence of parasitic tracking of the most common clone. Likewise, in a second pool where MML/I was only 4% more common than MML/II (pool 5), MML/I was still significantly more heavily parasitized than either MML/II or *P. monacha*.

There are two possible explanations for the differences between the current results and those of Lively et al. (1990). The first concerns the finding that MML/II is a combination of several histocompatibility types, whereas MML/I is made up of only one histocompatibility type (Moore and Eisenbrey 1979). Therefore, if differences in histocompatibility types are correlated with susceptibility to infection from this parasite, MML/I might still have been the most common susceptibility type in pools 5 and 7. This alternative is strengthened by the observation that MML/II had the highest variance in parasitic intensity of the three strains in pool 7 and a somewhat higher variance in pool 5. However, if the variance in parasitic intensity truly reflects variation in the number of susceptibility types in a strain, then *P. monacha* should have been the most variable of all three strains. Since this was not the case, it is unlikely that the variation in parasitic intensity is an indicator of the number of susceptible types within MML/II. Also, if a high number of susceptibility types within MML/II explains the lack of evidence for parasitic tracking of common genotypes in this study, then one must assume that the MML/II in Lively et al.'s (1990) sample was primarily composed of one of these susceptibility types, which would account for the observed reversal of parasitism recorded in their study. Unfortunately, without genetic markers to distinguish these susceptibility types, any experiments designed to quantify the number of these types within MML/II would require that large numbers of these fish be transported live into a laboratory setting for tissue grafting experiments (see Moore and Eisenbrey 1979).

A second possible explanation is that the reversal of parasitism reported by Lively et al. (1990) was not caused by the coevolutionary processes outlined in the RQ model but was probably due to one or more unrelated processes. A major problem of applying the RQ model to the *Poeciliopsis* system concerns the ability of the parasite to track their hosts over the distances reported by Lively et al. (1990). The parasites probably do not track their hosts so tightly as to follow individuals in a single pool but more likely track an average population over space and time. *Uvulifer* spp. are strigeoid trematodes with complex life cycles (Schmidt and Roberts 1985). The adult stage of this trematode infects fish-eating birds (e.g., kingfishers). Eggs produced by these adults hatch into miracidia that infect snails in the genus *Helisoma*. Cercariae develop in the snail hosts and are released into the water column where they then infect passing fish by encysting in their dermis. The life cycle is completed when the fish are eaten by a foraging bird. This life cycle creates two potential problems for operation of the RQ model over the distances separating the pools sampled in this stream. First, because the relative clonal composition of each isolated pool is unlikely to be maintained during the rainy season, the parasites essentially have a single dry season in which to adapt to the most common clone within any one pool. Because completion of the life cycle requires several steps, it is unlikely that enough generations are possible for the parasites to adapt to the most common genotypes in these local pools. Second, the tight host tracking outlined by Lively et al. (1990) requires genetic isolation of the parasite over distances of no more than several hundred metres. Because the activities of the parasites' primary host, piscivorous birds, undoubtedly spread the parasites over much greater distances than this, such local genetic isolation in the parasite populations is unlikely. Both of these qualifications indicate that the operation of the processes outlined in the RQ model in this reproductive complex on such a localized scale is doubtful.

One of two alternative hypotheses is more likely to explain the observation that MML/I is the most heavily parasitized fish strain in this complex. It is probable that selective pressures on the parasites are averaged across a series of evolutionarily interconnected pools. It is possible that the parasite is selectively infecting MML/I because it is the most common genotype across this series of pools, which would be consistent with the RQ model. In this scenario, the parasite would be tracking the most common genotype across a larger geographical range, which would explain the higher frequency of parasitism for MML/I across all of the Platanos. However, this alternative hypothesis remains to be confirmed.

A second explanation is that MML/I is inherently more susceptible to parasitism than the other two strains, regardless of its frequency. Such a situation has been described in a related clonal–sexual complex. Leberg and Vrijenhoek (1994) found that one of two hybridogenetic *P. monacha-lucida* clones was susceptible to infection by a different trematode parasite (*Gyrodactylus turnbulli*), while another coexisting clone was completely resistant. This trematode parasite does not cooccur with the fish in this hybridogenetic system, and thus there is no opportunity for coevolution among host and parasite in this particular example. Nevertheless, this example is interesting in that it underscores the possibility that different susceptibility genotypes can be frozen from a sexual population (Leberg and Vrijenhoek 1994). Such a difference in clonal susceptibility would cause a permanent parasitic bias for one clone over the other rather than the cyclic parasitic intensity outlined by the RQ model.

Distinguishing between these two possibilities requires either using a more intensive sampling regime that includes streams that can be considered as containing truly distinct parasite populations or, more conclusively, devising an artificial selective regime to demonstrate the tracking of hosts by these parasites under controlled conditions. Clearly, there is no conclusive evidence for tight parasitic tracking by these trematodes on the most common genotype in this reproductive complex.

Even if tracking of the most common genotype by the tre-

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matodes in this system is verified, there is no evidence that this parasite has a negative effect on fitness. In MML/I, the most heavily parasitized clone, there were two cases in which females had more than 225 parasitic cysts. These fish were almost completely black owing to the number of cysts in the epidermis. If parasitism negatively affects fitness (as assumed by the RQ model), then these fish should have either reduced fecundity or increased mortality. Nevertheless, one of these two had a size-corrected fecundity that was greater than average, while the other was smaller than average. In general, the relationship of size-corrected fecundity to parasite load was very weak. Thus, there is no indication that parasitism can create the reduced fecundity in the most common genotype that would be necessary to cause the type of coevolution envisioned in the RQ model. There is still a possibility that parasitism increases mortality, such as by making heavily parasitized fish more obvious to predators (Krause and Godin 1994) or by decreasing overwintering survival (Lemly and Esch 1984). These possibilities remain to be tested.

Nevertheless, clonal composition has remained reasonably stable for at least 90 generations in this reproductive complex (Schenck and Vrijenhoek 1989), with little evidence of the cycling in clonal dominance one would expect from the RQ model (Lively 1993). Although extensive sampling on a monthly basis over an extended period has not been undertaken, dozens of collections have been made over two decades at these sites (Vrijenhoek 1994). Overall, MML/I has remained the dominant clone in this stream over this time period, with MML/II always being in low abundance. This further suggests that the coevolutionary relationships between host and parasite predicted by the RQ model are either not present in this system or are so weak as to be ineffective at modifying clonal performance.

The genetic and ecological evidence so far collected from fish in this reproductive complex (see Vrijenhoek 1994) suggests that the long-term clonal and sexual coexistence of these lineages (Quattro et al. 1991) is best explained by resource or niche partitioning among strains, as predicted by the FNV model. At this point, the evidence for coevolutionary relationships between parasites and hosts remains equivocal, and the likelihood that the RQ model explains the coexistence of sexuals and clones in this system is doubtful.

Acknowledgments

I thank A. Varela and R. Schenck for help with the field collections; C. Craddock, F. Hensley, C. Lively, G. Meffe, and P. Niewiarowski for critically reviewing the manuscript; P. Dixon and K. Garrett for statistical advice; and R.C. Vrijenhoek for constructive criticism of the design of the study. This study was funded in part by a National Science Foundation doctoral dissertation support grant (BSR–8815423).

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Phylogeny of Pacific salmon and trout based on growth hormone type-2 and mitochondrial NADH dehydrogenase subunit 3 DNA sequences

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Abstract: The phylogeny of *Oncorhynchus* has previously been studied using a variety of morphological and genetic characters, but two unresolved problems remain: the position of masu (*Oncorhynchus masou*) and amago (*Oncorhynchus rhodurus*) salmon and the relationships within the group containing sockeye (*Oncorhynchus nerka*), pink (*Oncorhynchus gorbuscha*), and chum (*Oncorhynchus keta*) salmon. We examined relationships among nine *Oncorhynchus* species, Atlantic salmon (*Salmo salar*), and lake trout (*Salvelinus namaycush*) using DNA sequence analyses of the mitochondrial NADH dehydrogenase subunit 3 gene and a portion of the nuclear growth hormone type-2 gene. Phylogenetic trees inferred using both cladistic and distance approaches were highly concordant except in the placement of the outgroup; strong support is provided for the proposition that pink and chum salmon are sister species, and that masu and amago salmon are closer to the Pacific trout than the other Pacific salmon. The phylogeny inferred by total evidence cladistic analysis of our data combined with five different morphological, biochemical, and DNA character sets provides evidence that the common ancestor of rainbow (*Oncorhynchus mykiss*) and cutthroat (*Oncorhynchus clarki*) trout was the first to diverge from the proto-*Oncorhynchus* evolutionary line, which then radiated to form the seven extant Pacific salmon species.

Résumé : Divers chercheurs ont étudié antérieurement la phylogénie du genre *Onchorhynchus* en se fondant sur divers caractères morphologiques et génétiques. Toutefois, deux questions demeurent à ce jour sans réponse : la position des saumons masou (*Oncorhynchus masou*) et amago (*Oncorhynchus rhodurus*) au sein du genre et les liens phylogéniques au sein du groupe renfermant les saumons rouge (*Oncorhynchus nerka*), rose (*Oncorhynchus gorbuscha*) et kéta (*Oncorhynchus keta*). Nous avons examiné les relations entre neuf espèces du genre *Oncorhynchus*, le saumon atlantique (*Salmo salar*) et le touladi (*Salvelinus namaycush*), par séquençage du gène encodant la sous-unité 3 de la NADH-déshydrogénase mitochondriale et une portion du gène responsable de l'hormone de croissance nucléaire de type 2. Les arbres phylogénétiques produits à l'aide de l'approche cladistique et de l'approche des distances étaient très semblables, mis à part la place réservée au groupe isolé; de nombreux indices nous portent à croire que les saumons rose et kéta sont des espèces soeurs et que les saumons masou et amago sont plus près des truites de la côte du Pacifique que le saumon rouge. La phylogénie déduite à partir des analyses cladistiques de l'ensemble de nos données et de cinq ensembles différents de caractères morphologiques, biochimiques et génétiques démontrent que l'ancêtre commun de la truite arc-en-ciel (*Oncorhynchus mykiss*) et de la truite fardée (*Oncorhynchu clarki*) a été le premier à diverger de la lignée évolutive proto-*Oncorhynchus*, qui s'est à son tour dissociée pour former le groupe des sept espèces de saumons du Pacifique. [Traduit par la Rédaction]

Introduction

Historically, the presumed relationships among the Pacific salmon and trout have been the subject of some debate. Taxonomies based on morphology and life history previously placed rainbow (*Oncorhynchus mykiss*) and cutthroat (*Oncorhynchus clarki*) trout with Atlantic salmon (*Salmo salar*) and brown trout (*Oncorhynchus trutta*) in the genus *Salmo*. More recent work has led to the reclassification of rainbow and cutthroat trout as *Oncorhynchus* species (reviewed by Smith and Stearley 1989). The genus *Oncorhynchus* contains all Pacific salmon species, including masu (*Oncorhynchus masou*) and amago (*Oncorhynchus rhodurus*) salmon, which are found only in Asia. *Oncorhynchus* is believed to have arisen from a single ancestral species derived from the *Salmo* evolutionary line. Neave (1958) proposed that the common ancestor of rainbow and cutthroat trout was the first to diverge from the proto-*Oncorhynchus* evolutionary line, which then radiated to form the seven extant Pacific salmon species.

Oncorhynchus phylogenies have been reconstructed from morphology, physiology and ontogeny, DNA–DNA hybridization, protein electrophoretic mobility variation, karyology,

Received July 19, 1995. Accepted November 20, 1995.
J13008

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Table 1. Sequences used in this study.

Species	Common name	Origin[a]	Locus	Accession No.
O. clarki	Cutthroat trout	Coastal Cutthroat, Vancouver Island, B.C.	*GH2*[a]	GenBank U28156
			ND3[b]	NS
O. mykiss	Rainbow trout		*GH2*[c]	GenBank J03797
			ND3[b]	NS
O. tshawytscha	Chinook salmon	Chilliwack Hatchery, B.C.	*GH1*[d]	NS
			GH2[a]	GenBank U28157
			ND3[b]	NS
O. kisutch	Coho salmon	Chilliwack Hatchery, B.C.	*GH2*[a]	GenBank U28359
			ND3[b]	NS
O. nerka	Sockeye salmon		*GH1*[e]	GenBank U14551
			GH2[e]	GenBank U14535
			ND3[b]	NS
O. gorbuscha	Pink salmon	Weaver Creek Hatchery, B.C.	*GH2*[a]	GenBank U28360
			ND3[b]	NS
O. keta	Chum salmon	Weaver Creek Hatchery, B.C.	*GH2*[f]	GenBank L04688
			ND3[a]	GenBank U28365
O. masou	Masu salmon	Hokadate, Japan	*GH2*[a]	GenBank U28361
			ND3[a]	GenBank U28364
O. rhodurus	Amago salmon	Tamaki, Japan	*GH2*[a]	GenBank U28362
			ND3[a]	GenBank U28363
Salmo salar	Atlantic salmon	Cultured, Sea Spring Salmon Farm, Chemainus, B.C.	*GH1*[g,h]	EMBL X61938
			GH2[i]	GenBank M21573
			ND3[a]	GenBank U28366
Salvelinus namaycush	Lake trout	Moberly Lake, B.C.	*GH2*[a]	GenBank U29954

Note: NS, taken from reference and not located in data-base search.
[a]This study.
[b]Thomas and Beckenbach (1989).
[c]Agellon et al. (1988).
[d]Du et al. (1993).
[e]Devlin (1993).
[f]X. Shen, Y. Wang, M. Wett, D. Liu, and F.C. Leung, unpublished data.
[g]Lorens et al. (1989).
[h]Male et al. (1992).
[i]Johansen et al. (1989).

and DNA polymorphism and sequence analyses (Utter et al. 1973 and references therein; Berg and Ferris 1984; Thomas et al. 1986; Thomas and Beckenbach 1989; Grewe et al 1990; McVeigh and Davidson 1991; Phillips and Pleyte 1991; Shedlock et al. 1992, Devlin 1993; Murata et al. 1993; Takasaki et al. 1994). However, ambiguities still exist regarding the origins of masu and amago salmon, the branching order for more ancient lineages, and the more recent speciation events involving sockeye (*Oncorhynchus nerka*), pink (*Oncorhynchus gorbuscha*), and chum (*Oncorhynchus keta*) salmon.

DNA sequences of some nuclear growth hormone type-2 (*GH2*) and mitochondrial NADH dehydrogenase subunit 3 (*ND3*) genes have been examined previously in salmonid species (Table 1). In this study, we have sequenced a portion of the *GH2* locus from an additional seven species and the complete *ND3* gene of four species, making it possible to examine the relationships among the Pacific trout and all extant salmon species. We have compared our inferred phylogenies with those of other studies to address some of the remaining problems in the systematics of *Oncorhynchus*.

Materials and methods

Sample collections

Species used in this study are listed in Table 1. DNA from chum, amago, masu, and Atlantic salmon liver samples were used to obtain sequences from the *ND3* locus. *GH2* sequences were amplified from cutthroat trout, chinook (*Oncorhynchus tshawytscha*), coho (*Oncorhynchus kisutch*), pink, masu, and amago salmon, as well as lake trout (*Salvelinus namaycush*). Several *Oncorhynchus* species have a *GH2*-like Y chromosome linked pseudogene (Du et al. 1993; Forbes et al. 1994; R.H. Devlin, unpublished data). To avoid unintentional amplification of the male-specific *GH* pseudogene, DNA was isolated from the livers of female fish wherever possible. (The sex of the *Salvelinus namaycush* sample is unknown.)

DNA extraction and gene amplification

DNA was extracted from liver tissue according to the method of Devlin (1991). The concentration of DNA samples was determined with a Hoeffer DNA fluorometer. The polymerase chain reaction (PCR) and sequencing primers used (based on consensus sequences of salmonid species) are listed in Table 2 and their map positions are shown in Fig. 1.

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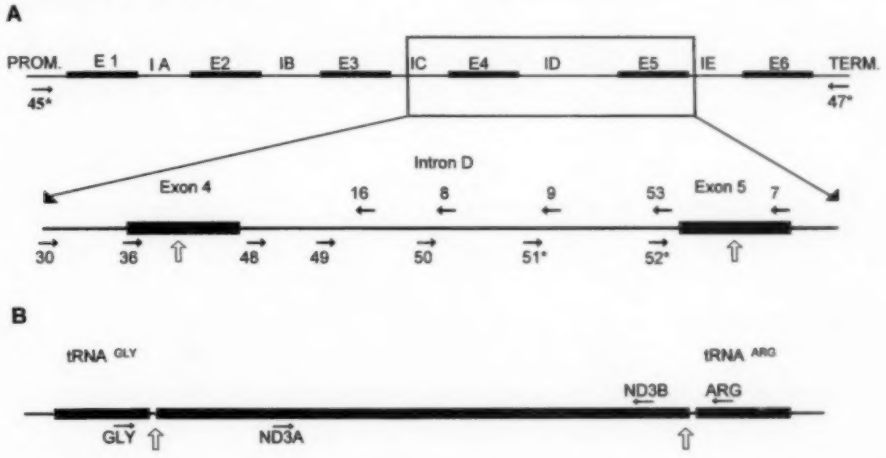
Table 2. Oligonucleotide primer sequences.

Primer	Sequence (5′ to 3′)
GH7	CTTATGCATGTCCTTCTTGAA
GH8	TGTGGCCTTCAAGTGAATTC
GH9	TATACAGAATCTGACTGCAG
GH16	TTGTTAATCTTTGTGAAAA
GH30	TTTCTCTACGTCTACATTCT
GH36	GTCCTGAAGCTGCTCCG
GH45[a]	GTACGCGGCCGCC(C/G)GAACTCATGGAAAAATTC *Not*I
GH47[a]	GTACGCGGCCGCATGTACTAATCTAAAATGTC *Not*I
GH48	CAAT(G/T)ACCATTTGTGGT
GH49	CA(C/T)GCTCTACTACAGGTA
GH50	AC(A/G)CCTCAAAATA(A/C)GG(C/T)C
GH51	GTCAAGCTGATACAACTC
GH52	AGTGAAATACAACTATGC
GH53	ACAGAGAGAGATCGATGG
ARG[a]	ATGCGGATCCT(T/C)TTGAGCCGAAATCA *Bam*HI
GLY[a]	ACGTGAATTCGTA(T/G)(A/G)(A/C)GTG(A/G)CTTCCAA *Eco*RI
ND3A	AAAT(C/T)TC(C/T)CC(A/C)GACGCA
ND3B	CATTCTAAGCCTCCTTGGG

[a]The four nucleotides at the 5′ end plus the *Not*I, *Bam*HI, and *Eco*RI restriction sites (underlined) are not present in the template sequence.

Fig. 1. Map of the locations of *GH2* and mitochondrial *ND3* gene amplification and sequencing primers. The position of each primer is represented by horizontal arrows. Sequenced regions are delimited by open, vertical arrows. (A) Growth hormone loci. E1–E5 are exons and IA–IE are introns. Primers were designed from aligned *GH1* and *GH2* sequences, except for those marked with asterisks, which are *GH2* specific. (B) Mitochondrial *ND3* sequence primers.



PCR amplifications were carried out in 25–100 μL volumes containing 1× PCR buffer (based on Medium buffer (Idaho Technologies) but with 1.5% (w/v) Ficoll), 6 ng/μL template DNA, 0.025 units/μL *Taq* polymerase (Bethesda Research Laboratories), 200 μM of each deoxynucleoside triphosphate (dNTPs), and approximately 1 pmol/μL of each amplification primer. PCR amplifications were carried out primarily in a Perkin Elmer 9600 thermal cycler. Some amplifications were also carried out on Biometra and Idaho Technologies thermal cyclers. PCR amplifications were performed with 30 cycles. Denaturation, annealing, and extension times were varied according to the thermal cycler used and the size of the expected amplification product.

Primers (*GH45* and *GH47*), designed to specifically amplify the *GH2* gene, were based on conserved sequences from the promoter and terminator regions identified by the alignment of all available *GH* sequence data from several salmonid species. Other *GH* sequencing

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and PCR primers (Fig. 1, Table 2) were designed based on intron D and flanking sequences of sockeye salmon *GH1* and *GH2* and, in the case of *GH48–53*, based on the alignment of all previously published *GH2* intron D sequences.

Multiple amplification products were often observed when using *GH* primers with a genomic DNA template. To isolate *GH2*-specific sequences, a portion of the complete *GH2* PCR product (from *GH45* and *GH47*) was reamplified using internal primers *GH7* and *GH30*, or *GH7* and *GH36*. These reamplification products were compared with the amplification products from a genomic DNA template using agarose gel electrophoresis. In each case, the *GH30* or *GH36* and *GH7* product amplified from *GH2* had the same electrophoretic mobility as one of the genomic DNA amplification products (data not shown). Wherever possible, the genomic DNA amplification product corresponding to *GH2* was isolated for cloning. In the case of chinook salmon, where the *GH2*-specific product could not be unambiguously distinguished from that of *GH1* using agarose gel electrophoresis, the *GH7–GH30* product reamplified from the *GH2* PCR product was cloned.

A mitochondrial DNA fragment containing *ND3* was amplified using primers (ARG and GLY) based on conserved regions of the genes for $tRNA^{Arg}$ and $tRNA^{Gly}$, which flank *ND3* in vertebrate mitochondrial genomes. To facilitate the sequencing of *ND3* from Atlantic salmon, for which the ARG primer worked poorly, the internal primers *ND3A* and *ND3B*, based on the alignment of all *Oncorhynchus ND3* sequences, were subsequently designed (Table 2, Fig. 1).

DNA cloning and sequencing

PCR amplification products to be cloned were purified by electrophoresis in agarose with a low melting point (Nusieve-GTG, FMC Biochemical), followed by isolation of DNA from excised bands using the Magic or Wizard PCRprep kits (Promega). The *ND3* and *GH2* amplification products were blunt-end cloned into pCRscript, a pBluescript derivative, using the pCRscript cloning kit (Stratagene).

The clones were sequenced on both strands using the single- and double-stranded methods described in the Sequenase 2.0 sequencing kit (United States Biochemical Corp.). Various combinations of the primers described in Fig. 1 and Table 2 were used in sequencing reactions. To compensate for the inherent error rate of *Taq* polymerase (Saiki et al. 1988; Tindall and Kunkel 1988; Keohavang and Thilly 1989) and possible differences owing to allelism in heterozygous individuals, a minimum of two clones were sequenced for each species. Sequence differences between clones (usually single nucleotide differences) were encountered at a rate of about 1 per 520 bases. Ambiguities were resolved by direct sequencing of PCR products or by sequencing the region in question from a third clone and accepting the consensus between two of the three sequences. Raw sequence data were processed and assembled using PC\Gene (Intelligenetics, Mountain View, Calif.). The final DNA sequences have been submitted to GenBank. (Accession numbers are listed in Table 1.)

Sequence and phylogenetic analysis

In addition to the sequences determined in this study, published sequence data from other species (Table 1) were incorporated into the data sets. Sequences were manually aligned using the Eyeball Sequence Editor (ESEE version 1.09d; Cabot and Beckenbach 1989). The sequenced *GH2* fragment contained intron D plus 100 (nucleotides, nt) each of 5′ and 3′ flanking exon sequence. The complete *ND3* coding sequence was determined.

Both cladistic and distance approaches to phylogeny reconstruction were used in this study to evaluate the consistency among methods and to determine the best estimate of the true phylogeny. Parsimony analysis was performed using the DNAPARS program of the PHYLIP version 3.5 package (Felsenstein 1993). The bootstrap analysis (2000 replicates) was performed with the taxon input order randomized once for each replicate. Neighbor-joining bootstrap trees (Saitou and Nei 1987) were constructed from Kimura two-parameter (Kimura 1980) corrected distance matrices using the NEIGHBOR program in PHYLIP version 3.5. Maximum likelihood analysis was performed with DNAML in the PHYLIP package. To search for the best tree, the global rearrangement option was selected and the taxon input order was randomized $n + 1$ times, where n is the number of taxa. To compare the likelihoods of alternative tree topologies, the user-defined tree option was selected. With this option, DNAML performs a statistical analysis to determine whether the likelihoods of alternative trees are significantly worse than that of the best, or maximum likelihood, tree (Kishino and Hasegawa 1989).

For phylogenetic analysis, only portions of the sequences that could be unambiguously aligned for all taxa were used. Gaps introduced to maximize alignment of the *GH2* intron sequence alignment were reduced to one site. Gaps were retained as single sites rather than completely removed because several of the 1-nt gaps remaining after reduction were phylogenetically informative (discussed below). Normally, gap sites can be scored as a character state in parsimony analysis but are ignored when calculating distance measures. To ensure that exactly the same data were considered with all methods of phylogeny reconstruction, each of the reduced gap sites was weighted as equivalent to one transitional (T ↔ C) change for both distance and parsimony analyses. The 100 nt each of flanking 5′ and 3′ sequence determined in this study was retained in the data set. The number of variable sites in the coding region was not substantially lower than that in the intron sequence (8 vs. 12.8%) and the ratios of transitions–transversions (discussed below) were similar for both types of sequence.

Total evidence (character congruence) cladistic analysis

The criteria for inclusion of each character set in this analysis were (*i*) availability of published data, (*ii*) completeness (only character sets that included at least six of the taxa in question were used), and (*iii*) relevance to the branching order of the (sockeye, pink, chum) clade (only data sets with all three taxa represented were used). The amago salmon taxon, which has appeared only rarely in other inferred *Oncorhynchus* phylogenies (Table 3), was not included in this portion of the analysis. Total evidence (Kluge 1989), or character congruence, analysis was performed on a pooled data set containing all synapomorphies from the *ND3* (52 characters) and *GH2* (18 characters) sequences identified in this study, as well as from morphological data (28 characters from Stearley and Smith 1993), protein variations (11 characters from Utter et al. 1973; 10 characters from Tsuyuki and Roberts 1963), mitochondrial DNA restriction site data (17 characters from Thomas et al. 1986), and sequence data (58 characters from Shedlock et al. 1992). The mitochondrial D-loop sequence had many alignment gaps. To avoid comparison of nonhomologous sites owing to alignment ambiguities, all insertion and deletion sites were removed from the data set. All data were converted to the same type by encoding character states from morphological data as 0–2 (as presented in Stearley and Smith (1993)); the presence or absence of a restriction site and protein electrophoretic mobility variant data as 0 and 1; and sequence data as G = 0, A = 1, T = 2, and C = 3. All character states were treated as unordered. The total evidence phylogeny was inferred using parsimony with the exhaustive search option in PAUP version 3.1 (Swofford 1993).

Results

Nuclear and mitochondrial DNA sequences

The nucleotide sequences of the mitochondrial *ND3* (Fig. 2) and a portion of the *GH2* (Fig. 3) gene for nine *Oncorhynchus* species, Atlantic salmon, and lake trout show DNA molecules that have undergone different processes of evolution, both in terms of nucleotide substitution rates and insertion and deletion events. From comparison of the pairwise distances be-

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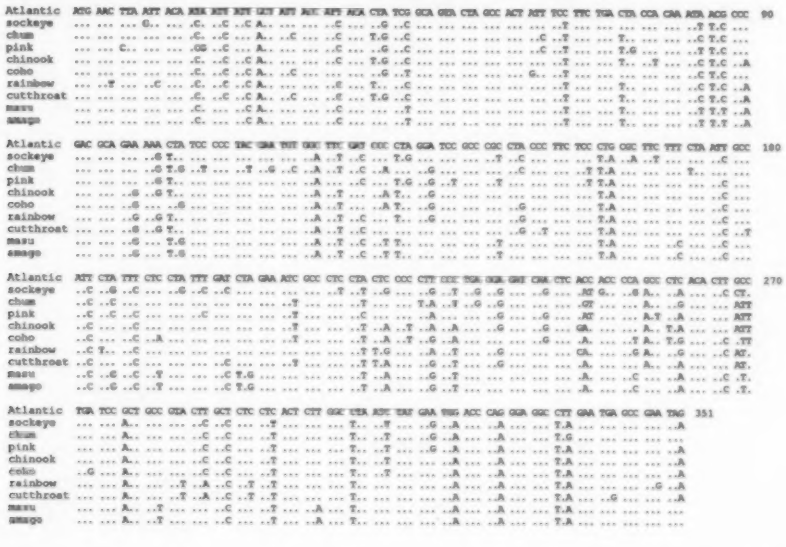
Table 3. Comparison of branching order of inferred *Oncorhynchus* phylogenies from this study with those of previous analyses.

Clades affected by node	Published studies	
	Supporting	Conflicting
(Atlantic, (masu, amago), (other *Oncorhynchus* spp.))	1	2,3,4,5,6,7
(Atlantic, ((sockeye clade),[a] (other *Oncorhynchus* spp.)))	2,7,8,17	1,3,4,5,6,9,10
((Coho, chinook), (sockeye clade))	1,2,3,5,6,7,8,9,10,11,12,13,14,15,16	4
(Chinook, coho)	1,2,3,6,7,8,10,11,12,13,14,15,16	4
(Sockeye clade)	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16	
(Pink, chum)	1,2,8,10,11,16	3,5,6,7,12,13,14,18
(Rainbow, cutthroat)	1,2,3,5,6,8,12,13,15	4
(Masu, amago)	1,2,3	

Note: 1, this study (*ND3*; Fig. 5A); 2, this study (*GH2*; Fig. 5B); 3, Smith and Stearley (1989); 4, Stearley and Smith (1993); 5, Shedlock et al. (1992); 6, Phillips and Pleyte (1991); 7, Hikita (1963); 8, Grewe et al. (1990); 9, Tsuyuki and Roberts (1963); 10, Murata et al. (1993); 11, Thomas et al. (1986); 12, Thomas and Beckenbach (1989); 13, Utter et al. (1973); 14, Tsuyuki and Roberts (1966); 15, Gorshkov and Gorshkova (1981); 16, Takasaki et al. (1994); 17, McVeigh and Davidson (1991); 18, Simon (1963).

[a]Denotes the (sockeye, pink, chum) clade without reference to internal branching order.

Fig. 2. Aligned DNA sequence of the mitochondrial *ND3* gene for 10 salmonid taxa. Codon triplets are separated by spaces. Dots indicate nucleotide identity with the initial sequence, Atlantic salmon. Species designations are listed in Table 1.



tween species (Table 4), it is clear that despite the fact that *ND3* is coding sequence and much of the *GH2* sequence is not, the rate of nucleotide substitution of the mitochondrial locus is considerably higher (an average of threefold). To compare the rate of change in coding sequence only, *ND3* distances from Table 4 were compared with their counterparts in a pairwise distance matrix (data not shown) calculated from the entire *GH2* coding sequence (630 nt) of rainbow trout and chum, sockeye, and Atlantic salmon (Table 1). The average ratio of pairwise percent differences in observed substitution rates in the mitochondrial *ND3* versus nuclear *GH2* coding sequences was 8.22 ± 1.74. A similar comparison with the noncoding *GH2* intron D and *ND3* sequences revealed a ratio of 4.16 ± 0.3.

The *GH2* segment and *ND3* data sets also differed in the transition (ti) to transversion (tv) ratios of observed nucleotide substitutions. The average ti/tv ratio in pairwise comparisons of the portion of *GH2* sequence obtained in this study was 1.41 within intron D and 1.33 in the flanking exon sequences. In the intron sequence, the ti/tv ratio ranged from 0.67 between chinook and rainbow to 3.0 between masu and amago. The average ti/tv ratio of the *ND3* gene was considerably higher at 5.3, ranging from 2.5 between Atlantic and coho to 18 between rainbow and cutthroat. Most of the substitutions observed in the *ND3* sequence were at two- and four-fold degenerate sites.

Amago and masu salmon have an identical *ND3* DNA sequence for the individuals we characterized. In contrast, the otherwise less variable *GH2* sequence fragment differs by 0.68% between masu and amago (Table 4). To confirm this observation, a fragment (220 nt) of the *ND3* gene was sequenced from an additional three individuals each of masu and amago salmon. With the exception of a single A ↔ G transi-

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Fig. 3. Aligned nucleotide sequence of a portion of the *GH2* locus from 11 salmonid taxa, comprised of intron D and portions of flanking exons. Dots indicate nucleotide identity with the initial sequence, Atlantic salmon. Dashes represent gaps introduced to produce optimal sequence alignment. There are 100 nt at each of the 3′ and 5′ ends that are coding sequences from exons four and five, respectively.

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Table 4. Pairwise Kimura two-parameter distance comparisons (%) based on sequence data.

	Sockeye	Chum	Pink	Chinook	Coho	Rainbow	Cutthroat	Masu	Amago	Atlantic
Sockeye		13.5	9.24	10.9	12.3	11.3	10.9	1.6	11.6	20.5
Chum	1.88		10.0	10.2	13.1	13.0	11.6	16.8	16.8	19.3
Pink	2.23	1.36		8.55	13.1	12.7	12.0	15.0	15.0	19.0
Chinook	1.71	2.93	3.28		6.31	7.57	6.93	11.7	11.7	18.0
Coho	3.10	4.35	4.34	2.40		11.2	10.6	11.3	11.3	17.2
Rainbow	2.40	3.63	3.98	2.05	3.81		5.71	11.3	11.3	18.8
Cutthroat	1.88	3.10	3.45	1.53	2.92	1.86		10.6	10.6	18.1
Masu	2.58	3.63	4.16	2.23	3.99	2.90	2.40		0.00	16.1
Amago	2.23	3.28	3.81	1.88	3.63	2.55	2.05	0.68		16.1
Atlantic	4.90	5.99	5.98	4.72	6.36	5.25	5.07	5.81	5.44	
Lake	3.82	5.07	5.07	3.82	5.44	4.17	4.17	4.71	4.35	3.65

Note: *ND3* distances are given above the diagonal and *GH2* distances, below. *GH2* distances were calculated from the sequence used in phylogenetic analysis: all gaps were reduced to one site and weighted equivalent to one T ↔ C transition.

Fig. 4. Insertion or deletion sites in *GH1* and *GH2* intron D sequences. Dashes represent gaps introduced to produce optimal sequence alignment. The presence of rearrangements specific to the *GH1* or *GH2* isoforms reveals that the two loci have been separate since before the divergence of Pacific and Atlantic salmonids.

```
Atlantic  GH2 AGTTGAAGTCA--GTCAATGAAA...//...TCTAAATGAG---TCACATTAAT
lake      GH2 AGTTGAAGTCA--GTCAATGAAA...//...TCTAAATGAG---TCACATTAAT
sockeye   GH2 AGTTGAAGTCA--GTCAATGAAA...//...TCTAAATGAG---TCACATCAAT
chum      GH2 AGTTGAAGTCA--GTCAATGAAA...//...TCTAAATGAG---TCACATCAAT
pink      GH2 AGTTGAAGTCA--GTCAATGAAA...//...ACTAAATGAG---TCACATCAAT
chinook   GH2 AGTTGAAGTCA--GTCAATGAAA...//...TCTAAATGAG---TCACATCAAT
coho      GH2 AGTTGAAGTCA--GTCAATGAAA...//...TCTAAATGAG---TCACATCAAT
rainbow   GH2 AGTTGAAGTCA--GTCAATGAAA...//...TCTAAATGAG---TCACATCAAT
cutthroat GH2 AGTTGAAGTCA--GTCAATGAAA...//...TCTAAATGAG---TCACATCAAT
masu      GH2 AGTTGAAGTCA--GTCAATGAAA...//...TCTAAATGAG---TCACATCAAT
amago     GH2 AGTTGAAGTCA--GTCAATGAAA...//...TCTAAATGAG---TCACATCAAT
chinook   GH1 AGTTGAAGTCAAGGTCAATGAAA...//...ACTAAATGAGAAGTCACATCAAT
Atlantic  GH1 AGTTGAAGTCAAAGTCAATGAAA...//...ACTAAATGAGAAGTGACATCAAC
sockeye   GH1 AGTTGAAGTCAAAGTCAATGAAA...//...ACTAAATGAGAAGTCACATCAAT
```

tional polymorphism (nt position 205) in the masu salmon *ND3* gene, the sequences were identical in all individuals. A more extensive study is required to determine whether recent introgression of the mitochondrial genome into one of these species has occurred.

Insertion–deletion patterns in *GH* intron D

The total aligned length of the *GH2* sequence fragments used in this study was 1406 nt. Individual sequences ranged from 635 to 1376 nt in length as a result of numerous insertion–deletion sites (Fig. 3). *GH1* and *GH2* are paralogous genes, presumably resulting from the tetraploidization of the ancestral salmonid genome (Ohno 1970; Allendorf and Thorgaard 1984). The *GH1* and *GH2* lineages are clearly distinct, and the two genes display little evidence of recent intergenic recombination after their divergence (Devlin 1993). This is consistent with the fact that several deletion sites are common to all *GH2* intron sequences of *Oncorhynchus* species examined here, but absent in the *GH1* introns from chinook, Atlantic, and sockeye salmon (Fig. 4).

Gaps revealed by sequence alignment of the introns show that such events are common in the evolution of these noncoding sequences (Devlin 1993). Shared, derived (synapomorphic) deletions of identical length and position involving two or more, but less than $n - 2$, taxa can be used as phylogenetically informative character states. The single large gap of over 700 nt common to masu and amago salmon (Fig. 3, nt positions 530–1244) is an example of such an informative event. Pink and chum salmon also share gaps not present in other taxa (nt positions 343, 1011–1272), supporting a close relationship between the two species. Several deletions were shared by all *Oncorhynchus* species examined here (e.g., nt positions 109–121 and 54–70) but were absent in lake trout and Atlantic salmon, indicating that the absence of this deletion is the plesiomorphic, or more primitive, state.

Phylogenetic inference using *GH2* and *ND3* sequences

To evaluate consistency among methods and between data sets, three approaches to phylogeny reconstruction (parsimony, maximum likelihood, and neighbor-joining distance analyses) were used. The Kimura two-parameter method (1980) was used for distance calculations. For each data set, all three methods produced trees with the same topology. With the exception of the placement of the outgroup clade, the *GH2* consensus tree topology was the same as that of *ND3* (Fig. 5).

Bootstrap testing was performed with 2000 replicates for both the neighbor-joining and parsimony methods. The bootstrap confidence levels, shown at the nodes in phylogenetic trees, represent the percentage of replicates in which that particular node or branch point occurred. The bootstrap values tended to be higher at terminal nodes, providing support for the species' pairs (chinook, coho), (masu, amago), and (cutthroat, rainbow) and the group (sockeye (pink, chum)). The consistent monophyly observed with (rainbow, cutthroat) and (chinook, coho) clades is also well supported by previous phylogenetic analysis (Table 3). Although the grouping (sockeye (pink, chum)) is supported by bootstrap analysis, particularly of the *GH2* data, it conflicts with some of the previously published inferred phylogenies (Table 3).

To resolve the rooting of the *Oncorhynchus* phylogenetic tree and address ambiguities in the systematics of the sockeye salmon clade, data from other studies were used in combination with our data sets to construct a total evidence (Kluge 1989) estimate of the species' phylogeny. Seven independent character sets were used, including the *ND3* and *GH2* data in this study as well as data from five previously published studies (Tsuyuki and Roberts 1963; Utter et al. 1973; Thomas et al.

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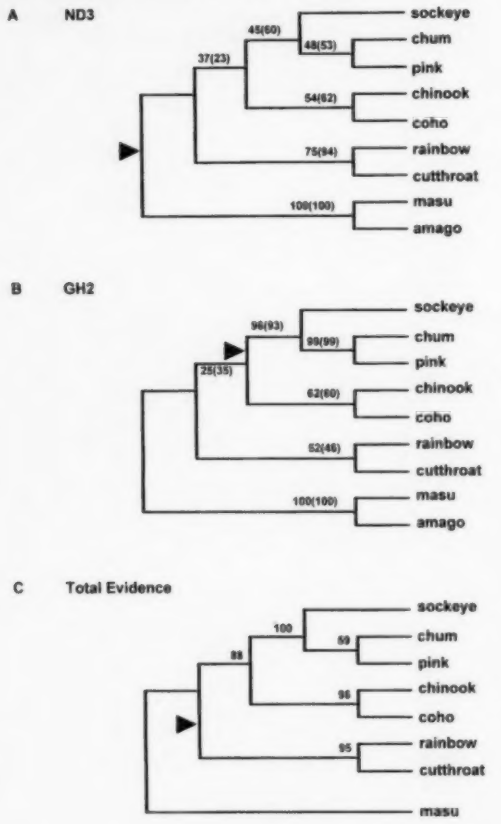
Fig. 5. Unrooted trees indicating the relationships among *Oncorhynchus* species. The arrow indicates the position where the branch leading to the outgroup taxa joins the tree. Parsimony and neighbor-joining (in parentheses) bootstrap confidence levels are given at the relevant nodes. Consensus phenogram of neighbor-joining, parsimony, and maximum-likelihood trees inferred from (A) *ND3* nucleotide sequences with Atlantic salmon as the outgroup and (B) *GH2* nucleotide sequences with Atlantic salmon and lake trout as the outgroup. The latter constituted a monophyletic clade distinct from *Oncorhynchus*. (C) Total evidence cladistic analysis: one most parsimonious tree was inferred on the basis of 183 synapomorphies from seven different morphological, biochemical, and DNA character sets.



1986; Shedlock et al. 1992; Stearley and Smith 1993). Except for the placement of the outgroup root, the total evidence tree had the same topology as the others shown in Fig. 5. Maximum likelihood analysis detected no significant difference (Kishino and Hasegawa 1989) in the log likelihood values of the three alternative trees from Fig. 5 with either of the *ND3* or *GH2* data sets.

Discussion

Mode and tempo of change in the *ND3* and *GH2* loci

The average ratios of observed pairwise distances between mitochondrial coding versus nuclear coding sequences (8.22 ± 1.74; *ND3* vs. *GH2* exons) and mitochondrial coding versus nuclear noncoding sequences (4.16 ± 0.30; *ND3* vs. *GH2* intron D) are consistent with the generally accepted higher rate of change in mitochondrial DNA. It is also important to note that the observed mitochondrial sequence divergence rates are probably underestimates, particularly when comparing more distantly related taxa, as the limited number of nucleotide sites that are free to vary become saturated for changes. The higher rate of substitution in mitochondrial DNA is thought to be due to the inefficient mismatch-repair mechanism in mitochondrial DNA replication (Brown et al. 1979). The twofold lower ratio of *ND3* versus *GH2* intron D probably reflects the absence or reduction of selective pressure on the noncoding intron sequence, allowing a higher rate of substitution relative to the adjacent coding regions.

The ti/tv ratio was consistently higher among *ND3* sequences than among those of *GH2* (an average of 5.1 vs. 1.33–1.41). This observation is also consistent with the generally accepted higher ti/tv ratios in mitochondrial DNA and suggests that mismatch at DNA replication, the primary mechanism of mutation in mitochondrial DNA sequence, favors transitional versus transversional substitutions. For both data sets, the ti/tv ratio tended to be much higher for closely related species' pairs and lower for more distantly related pairs.

Resolving the taxonomy of *Oncorhynchus*

The phylogenetic relationships among members of the genus *Oncorhynchus* have been the source of debate for a considerable period. Originally, the genus *Salmo* encompassed salmonid species from both Pacific and Atlantic drainages. Because of similarities between Pacific trout and Atlantic salmon in morphological characters, such as the number of anal fin rays and life histories, rainbow and cutthroat trout were retained in *Salmo* when Pacific salmon were classified into *Oncorhynchus*. However, increasing resolution of systematic analysis brought about by additional morphological and biochemical characters (reviewed by Smith and Stearley 1989) suggested a closer relationship to other Pacific salmonids, leading to the eventual placement of rainbow and cutthroat trout in *Oncorhynchus*.

In this study we have examined the phylogeny of the genus *Oncorhynchus* by comparing the genealogies of a nuclear (*GH2*) and a mitochondrial (*ND3*) locus. The rationale for examining both loci was to perform independent phylogenetic analyses to determine whether the conclusions were complementary. The genealogy reconstructed for a single genetic locus may not necessarily be taken as a representation of the phylogeny of the species from which the data were obtained. Biases introduced by the examination of sequence data from a single locus may cause inferred genealogies to differ among loci (Friedlander et al. 1994). Confounding influences, such as (*i*) differing rates of change of separate loci, lineages, or genomes, (*ii*) introgression owing to interspecific hybridization, and (*iii*) homoplasy owing to multiple substitutions at the same site, may play larger or smaller roles depending on the dynamics of local evolution of a particular locus. Another consideration is that the examination of only one representative from each species could introduce a bias if there were considerable intraspecific variation, particularly if the geno-

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type of the sampled individual resulted from past introgression or hybridization events.

The use of different approaches to phylogenetic reconstruction reduces the impact that biases inherent to particular methods can have upon the inferred phylogeny. Although self-consistency within a data set containing a strong phylogenetic signal will often support the same conclusions on the basis of different approaches to phylogenetic analysis (as was observed in this study), the condition of independence may not necessarily be satisfied by applying different methods of analysis to the same data. However, concordance between proposed trees based upon a variety of systems and genetic loci using both cladistic and distance approaches can be taken as an intuitive measure of confidence in a tree topology.

This work has been preceded by a number of other molecular phylogenetic studies of salmonid phylogeny based on mitochondrial DNA sequences (Thomas and Beckenbach 1989; Shedlock et al. 1992), growth hormone sequences (Devlin 1993), mitochondrial and nuclear restriction site differences (Phillips and Pleyte 1991; Thomas et al. 1986; Grewe et al. 1990), protein variations (Utter et al. 1973; Tsuyuki and Roberts 1963, 1966), and insertion patterns of short interspersed repetitive elements (SINEs; Murata et al. 1993; Takasaki et al. 1994). The groupings of species produced by terminal (more recent) and penultimate nodes in the consensus tree are all well supported by such analyses (Table 3): (pink, chum, sockeye), (chinook, coho), (rainbow, cutthroat), and (masu, amago) are all robust clades in terms of both statistical analysis and concordance with trees inferred from other molecular data.

Considering only *Oncorhynchus* species, the phylogenies reconstructed in this study were concordant not only between alternative methods of phylogenetic inference but also between two different genes. The consensus phylogeny supports a closer relationship between the Japanese salmon and Pacific trout than either group has with the other Pacific salmon species. This arrangement agrees well with a number of other, independent phylogenetic analyses (Table 3). Not considering the position of the root, the position of the Japanese salmon in the *GH2* and *ND3 Oncorhynchus* phylogenies is consistent with phylogenies proposed in Utter et al. (1973) and Phillips and Pleyte (1991) and suggested by Murata et al. (1993).

Rooting of the *Oncorhynchus* phylogenetic tree

To examine ancestral relationships and the branching order of more ancient lineages within the *Oncorhynchus* phylogeny, Atlantic salmon (*ND3* data and *GH2* data) and lake trout (*GH2* data) were used as outgroup taxa in phylogenetic analysis. The presence of a more distantly related outgroup in a phylogenetic reconstruction has the effect of polarizing the tree, making it possible to infer which taxa represent ancient lineages and which have diverged more recently. Introduction of Atlantic salmon to the phylogenetic analyses of *GH2* and *ND3* data produced trees that differed in the placement of the outgroup clade. Because of the discordance between the *GH2* and *ND3* trees, it was not possible to resolve the root of the phylogenetic tree on the basis of our data. To address this problem, we have used the concept of character congruence to determine the consensus of the *ND3*, *GH2*, and five additional independent character sets, with a view to finding the best estimate of the true phylogeny. Except for the position of the outgroup clade, the tree inferred by this approach was identical to the consensus of the *ND3* and the *GH2* trees. The outgroup rooting of the phylogenetic tree on the rainbow–cutthroat lineage does not agree with the trees reconstructed with either *GH2* or *ND3* sequence data (Fig. 5). However, statistical analysis with maximum likelihood (Kishino and Hasegawa 1989) using both *GH2* and *ND3* sequence data revealed that the rooting of the tree on the rainbow–cutthroat lineage was no less likely than either of the other branching orders observed here. Because numerous other reported branching orders also suggest that the rainbow–cutthroat lineage is the most ancient within *Oncorhynchus*, we favor this arrangement as the best estimate of the true phylogeny of this genus.

Uncertainty in the relationships among sockeye, pink, and chum salmon

The phylogenies inferred from the *GH2* and *ND3* data agree on the pairing of pink and chum salmon as sister species, and this conclusion is supported by the total evidence approach. To date, the systematic consensus has been to group sockeye and pink as sister species. This association is borne out by morphology (Smith 1992; Stearley and Smith 1993), karyology (Simon 1963; Gorshkov and Gorshkova 1981), and other character types (Table 3). Smith (1992) asserted that the conflicting evidence observed by Thomas et al. (1986) with restriction analysis of mitochondrial DNA and the similarities in the life histories of pink and chum salmon can be explained by introgression owing to hybridization. However, the phylogenetic trees observed in this study strongly support the branching order (sockeye (pink, chum)).

Two deletions in the *GH2* intron D were present in chum and pink but not sockeye salmon, providing unambiguous evidence that the *GH2* loci in these species are more closely related than either is to sockeye *GH2*. A closer relationship between these species has also been inferred by Murata et al. (1993) and Takasaki et al. (1994) based on species-specific or lineage-specific insertion of SINE repeat elements in the nuclear genome. Because both mitochondrial and nuclear DNA characters conflict with the pairing of sockeye and pink salmon, it would have been necessary for the putative introgression event or events between pink and chum salmon to have substituted blocks of homoplasic characters both in the nuclear as well as mitochondrial genomes. The hypothesis of historic introgression of the complete chum salmon mitochondrial genome into pink salmon (Smith 1992; Stearley and Smith 1993) conflicts with the conclusion of Shedlock et al. (1992) that pink and sockeye are sister species based on mitochondrial D-loop sequence data.

Because other closely related species' pairs within *Oncorhynchus* occupy similar niches without accumulating sufficient homoplasic morphological characters to confound phylogenetic inference to this degree (Table 3), convergence alone is not a satisfactory explanation to account for the large degree of discordance within and among character sets for pink, chum, and sockeye salmon.

Dating divergence events in *Oncorhynchus* evolution

On the basis of the analysis of fossil specimens found in Idaho (Smith 1992), pink, chum, and sockeye salmon have been separate and distinct species for at least six million years. Using salmon growth hormone sequences, Devlin (1993) esti-

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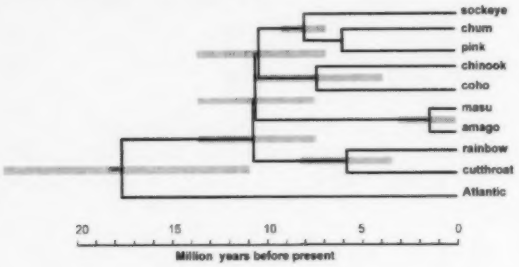
Fig. 6. The evolution of *Oncorhynchus* based on the inferred total evidence phylogeny. Shaded bars represent the range between time estimates based on *ND3* and *GH2* sequence divergence. In all cases, the *ND3* time estimate defines the low, or right-most end of the range. To prevent negative branch lengths between internal nodes, the mean time estimates for the (from left to right) second through fourth nodes for *GH2* and second and third nodes for *ND3* were used. The distance between these nodes was exaggerated to emphasize the inferred branching order.



mated that disomy in Salmonidae was established at least 27.2 million years ago, which is consistent with dating of a protosalmonid fossil (*Eosalmo driftwoodensis*) to the middle Eocene (Wilson 1977), and that Pacific and Atlantic salmonids diverged a minimum of 19.9 million years ago. Examination of the level of DNA sequence divergence observed in this study (Table 4) makes it possible to estimate the rate of divergence among *Oncorhynchus* species. For the purposes of these calculations, we assume that the age of the (pink, chum) species' pair is at least 6 million years. The *ND3* sequence divergence corresponding to this interval is 10%. Assuming a constant molecular clock, the accumulation rate of substitutions for *ND3* was estimated as (10/6)/2, or 0.833%/million years. This value is more than two times lower than the conventional 2%/million years clock rate for higher vertebrates. The mitochondrial genomes of poikilotherms have been shown to evolve at a lower rate than their mammalian counterparts (Martin and Palumbi 1993). A lower clock rate for salmon mitochondrial DNA is consistent with similar observations from Perciformes species (Cantatore et al. 1994) and turtles (Avise et al. 1992). Moreover, the lower rates observed in warm-blooded vertebrates such as cetaceans (Hoelzel et al. 1991) cast doubt on the concept of a universal molecular clock rate for higher vertebrates. The average pairwise distance between pink and chum salmon using the *GH2* sequence data is 1.36%, corresponding to a divergence rate of 0.113%/million years, considerably lower than the mitochondrial rate.

All rate estimates must be accepted with the caveat that they are vulnerable to violations of the assumption of a constant molecular clock. The validity of this assumption can be tested using a relative rate test (Sarich and Wilson 1973; Li et al. 1987). *Oncorhynchus* species are monophyletic with respect to Atlantic salmon. If the clock rate is constant between lineages, all taxa should be approximately the same distance from this outgroup. Since the level of DNA sequence divergence between pink and chum was used to calibrate the molecular clock, it is important to determine whether the average mutation rate in this lineage is equal to those of the other *Oncorhynchus* species. For the *ND3* sequence data (Table 4), the average pairwise distance between the (pink, chum) clade and Atlantic salmon is 19.15%, which differs by between – 0.135 and 3.05% from the corresponding distances for other taxa. Similarly with the *GH2* data, the average distance between (pink, chum) and Atlantic salmon was 5.99%, differing by between – 0.37 and 1.09% from the corresponding distances for the other taxa.

Among the more distantly related taxa, particularly with the *ND3* sequence, the clock rate may be underestimated because of homoplasy at nucleotide positions that are saturated for change. We feel that this effect has been minimized by (*i*) using the Kimura two-parameter method to perform distance calculations and (*ii*) calibrating the molecular clocks with the most recent divergence event for which a minimal time estimate is provided by the fossil record. For additional cautions on the use of molecular clock assumptions, see Moritz et al. (1987) and Hillis and Moritz (1990).

Applying the molecular clock estimates discussed above and bearing in mind their inherent limitations, we applied a crude time scale to the divergence or speciation events in *Oncorhynchus* phylogeny (Fig. 6). Time estimates were calculated with the formula $d/2k$, where d is the pairwise distance between taxa (or average distance between clades) and k is the molecular clock rate for that locus. The time estimates based on the clock rates of *ND3* and *GH2* differed, with the *ND3* estimates consistently lower. Our time estimates are consistently higher than those observed by Shedlock et al. (1992) using salmonid mitochondrial D-loop sequences. However, it should be noted that the estimates based on D-loop sequences were calculated using the mutation rate for mammalian D-loop sequences. When the D-loop data subset considered here was reanalyzed using the methods described above, the time estimates were either slightly lower than, or fell within, the range delimited by the *GH2* and *ND3* estimates. The same was true when estimates were calculated on the basis of the mitochondrial DNA distance data presented in Thomas and Beckenbach (1989).

On the basis of the mean of the divergence times calculated with *ND3* and *GH2* sequence data, we estimate that the minimum age of *Oncorhynchus*, or the time since it diverged from the ancestor it shares with *Salmo* and *Salvelinus*, is approximately 18 million years (Fig. 6). Some 8 million years later, the first in a rapid series of speciation or divergence events occurred, leading to the radiation of four main lineages, which in turn gave rise to the nine Pacific salmon and trout species. The distance between the second, third, and fourth internal nodes or branching points in the phylogeny was essentially zero (slightly exaggerated in Fig. 6 to show inferred branching order), indicating that the radiation leading to the four main clades was essentially instantaneous on this time scale. The fact that the early divergence events occurred over a very short period, combined with the lack of such radiation of the closely related genus *Salmo*, which occupies a similar range in the Atlantic basin, suggests that geologic or climatic conditions unique to the North Pacific basin opened up a new series of ecological niches, leading to the episodic bursts of speciation observed in the inferred *Oncorhynchus* phylogeny.

Because the time estimates are based on divergence rates of *ND3* and *GH2* sequences, the short interval over which the earlier divergence events occurred may have played a role in our failure to resolve the deeper phylogeny using these data

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alone. However, we have inferred the deeper branching order with the total evidence approach, using combined character sets selected from a variety of sources. The total evidence tree shows that the Pacific trout lineage is the most ancient within *Oncorhynchus*, which is also the consensus of previous phylogenetic inference for this genus.

Note added in proof

The combined data set used to infer the phylogeny represented in Figs. 5*c* and 6 was expanded with additional nuclear 18S rDNA restriction site (R.B. Phillips, K.A. Pleyte, and M.R. Brown. 1992. Can. J. Fish. Aquat. Sci. 49: 2345–2353) and mitochondrial ATPase 6 DNA sequence characters (M.J. Domanico and R.B. Phillips. 1995. Mol. Phylogenet. Evol. 4: 366–371). The single most parsimonious tree produced by reanalysis of the expanded data set has the same topology as that shown in the above figures.

Acknowledgments

The authors acknowledge D. Groves, F. Yamataki, I. Nakayama, and H. Onozato for provision of Atlantic and Japanese salmon samples; F. Breden for useful discussion; and the reviewers for helpful comments on the manuscript. This work was supported by Natural Science and Engineering Research Council of Canada (NSERC) grants to R.H.D. and M.J.S., an NSERC scholarship to S.J.M., and a Graduate Research in Engineering and Technology award to S.J.M. from the Science Council of British Columbia.

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Cytochrome P4501A1 induction by polychlorinated biphenyls (PCBs) in liver cell lines from rat and trout and the derivation of toxic equivalency factors

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Abstract: Toxic equivalency factors (potency of a compound relative to the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) standard) for polychlorinated biphenyls (PCBs) were derived from cytochrome P4501A1 induction, measured as ethoxyresorufin *o*-deethylase (EROD) activity, in a rainbow trout (*Oncorhynchus mykiss*) liver cell line (RTL-W1) and a rat hepatoma cell line (H4IIE). In RTL-W1 under normal growth conditions, EROD activity was induced only by PCBs 77, 81, 118, and 126. In overconfluent RTL-W1 cultures, EROD induction was also detected with PCBs 105, 156, and 169. Immunoblotting with the polyclonal antibody anti-trout CYP1A1$_{277-294}$/KLH confirmed the results of EROD induction. The RTL-W1 toxic equivalency factors were 0.023 for PCB 126, 0.0064 for PCB 81, 0.0034 for PCB 77, 0.000 16 for PCB 169, and 0.000 017 for PCB 118. Toxic equivalency factors for PCBs 105 and 156 were <0.000 03. All seven PCBs clearly induced EROD activity in H4IIE. Generally the H4IIE toxic equivalency factors were higher than the RTL-W1 toxic equivalency factors. Therefore, the toxic impact of PCBs on rainbow trout probably would be overestimated by currently proposed risk assessment toxic equivalency factors and assessed more appropriately with toxic equivalency factors derived from rainbow trout.

Résumé : On a déterminé la valeur du facteur d'équivalence toxique (ou pouvoir toxique d'un composé par rapport à un étalon de 2,3,7,8-tétrachlorodibenzo-*p*-dioxine (TCDD)) de divers biphényles polychlorés (BPC) d'après l'induction du cytochrome P4501A1, évaluée d'après l'activité de l'éthoxyrésorufine *o*-déséthylase (EROD) chez une lignée de cellules hépatiques de truite arc-en-ciel (*Oncorhynchus mykiss*) (RTL-W1) et chez une lignée de cellules d'hépatome de rat (H4IIE). En conditions de culture normales, l'activité EROD n'a été induite que par les BPC 77, 81, 118 et 126 dans les cellules RTL-W1; toutefois, dans des cultures surconfluentes de RTL-W1, les BPC 105, 156 et 169 ont aussi induit une activité EROD. L'immunotransfert avec des anticorps polyclonaux anti-CYP1A1$_{277-294}$/KLH de truite a confirmé ces observations. On a obtenu les facteurs d'équivalence toxique suivants dans les cellules RTL-W1 : 0,023 pour le BPC 126, 0,0064 pour le BPC 81, 0,0034 pour le BPC 77, 0,000 16 pour le BPC 169 et 0,000 017 pour le BPC 118. Le facteur d'équivalence toxique des BPC 105 et 156 était inférieur à 0,000 03. Dans les cultures de H4IIE, les sept BPC ont nettement induit une activité EROD. En général, les facteurs d'équivalence toxique mesurés dans les cellules H4IIE sont plus élevés que ceux obtenus avec les cellules RTL-W1. Par conséquent, avec les facteurs d'équivalence toxique qu'on propose actuellement d'employer dans les évaluations de risque, on surestimerait probablement l'effet toxique des BPC chez la truite arc-en-ciel; l'estimation devrait être plus juste avec des facteurs d'équivalence toxique déterminés au moyen de cellules de truite arc-en-ciel. [Traduit par la Rédaction]

Introduction

Toxic equivalency factors (TEFs) are a means for assessing the risk of polychlorinated biphenyls (PCBs) and of PCB mixtures (Barnes et al. 1991; Safe 1994). PCBs, of which 209 exist, are often found in the environment as complex mixtures of different PCBs and of other polyaromatic halogenated hydrocarbons (PHHs), such as polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). Like many PCDDs and PCDFs, some PCBs exert their toxic effects through the Ah (Aryl hydrocarbon) receptor. The Ah receptor both mediates the effects of these environmental contaminants and plays a role in the development of the liver and of the immune system (Fernandez-Salguero et al. 1995). The assumption that the toxicity of halogenated aromatic hydrocarbons is mediated through binding to an Ah receptor is the basis for a TEF approach to assessing relative toxicities of PCDDs, PCDFs, and PCBs (Safe 1990). A TEF is the fractional potency of a compound relative to a standard toxin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). TCDD is used as the

Received May 26, 1995. Accepted November 20, 1995.
J12932

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reference because this contaminant appears to be one of the most potent and has the most completely understood mechanism of action. The TEFs are used to convert analytical data for mixtures of PCDD, PCDF, and PCB into TCDD equivalents (TEQs). Concentrations of individual compounds in a complex mixture, such as an environmental sample, are multiplied by their TEFs and the result summed to give a single TEQ.

Although a variety of Ah-mediated end points have been measured in vivo and in vitro to derive TEFs for mammals, the in vitro approach provides a rapid, inexpensive way of screening large numbers of compounds and fulfils a societal desire to reduce the use of animals in research. The usual in vitro response has been the increased expression of the CYP1A1 gene (Bradlaw and Casterline 1979; Bosveld and Vanden Berg 1994). The result is enhanced levels or induction of the cytochrome P450 protein, P4501A1. This is assayed as aryl hydrocarbon hydroxylase (AHH) or 7-ethoxyresorufin *o*-deethylase (EROD) activity. The cells in which CYP1A1 induction is measured most commonly have been the rat hepatoma cell line H4IIE (Safe 1994).

Among PCBs, the non-ortho and mono-ortho coplanar PCBs exert actions through the Ah receptor (Safe 1994). The non-ortho coplanar PCBs are substituted in both para, at least two meta, and no ortho positions. Their most toxic members are 3,3′,4,4′-tetrachlorobiphenyl (PCB 77), 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126), and 3,3′,4,4′,5,5′-hexachlorobiphenyl (PCB 169). The mono-ortho coplanar PCBs are chlorinated at one ortho position. Some congeners of this class eliciting toxic responses are 2,3,3′,4,4′-pentachlorobiphenyl (PCB 105), 2,3′,4,4′,5-pentachlorobiphenyl (PCB 118), and 2,3,3′,4,4′,5-hexachlorobiphenyl (PCB 156). Both classes of PCBs bind to the Ah receptor and induce CYP1A1 gene expression.

The demonstration of the Ah receptor in a range of aquatic vertebrates (Hahn et al. 1994), including rainbow trout (*Oncorhynchus mykiss*), supports the development of TEFs for aquatic organisms and their use in aquatic environmental risk assessment. Early life stage mortality in rainbow trout (Walker and Peterson 1991), embryotoxicity in medaka (*Orizias latipes*) (Harris et al. 1994), and AHH or EROD induction in rainbow trout (Janz and Metcalfe 1991; Harris et al. 1994; Newsted et al. 1995; Parrott et al. 1995) are in vivo responses that have been used to develop piscine TEFs. The in vitro response has been the induction of EROD activity in cell lines from topminnow (*Poeciliopsis lucida*) hepatoma (PLHC–1) (Tillitt and Cantrell 1992; Hahn et al. 1993) and from rainbow trout liver (Clemons et al. 1994). Although relatively few species have been examined, and primarily with dioxins and furans, the results suggest that organism-specific TEFs might be appropriate for aquatic ecotoxicology (Grimwold and Dobbs 1995).

A limited amount of research intimates that the derivation of TEFs for PCBs could be more dependent on the species than is the case for other PHHs (Stegeman and Hahn 1994). In this report we have investigated this directly by comparing the ability of four non-ortho coplanar PCBs and three mono-ortho coplanar PCBs to induce EROD activity in the rat cell line H4IIE and in the trout cell line RTL-W1 and have used this information to derive TEFs. Confirmation of CYP1A1 induction in RTL-W1 has been sought by Western blotting with the polyclonal antibody anti-trout $CYP1A1_{277-294}$/KLH to rainbow trout P4501A1 (Myers et al. 1993).

Methods

Chemicals

The PCB congeners 3,3′,4,4′- (PCB 77) and 3,4,4′,5-tetrachlorobiphenyl (PCB 81); 2,3,3′,4,4′- (PCB 105), 2,3′,4,4′,5- (PCB 118), and 3,3′,4,4′,5,-pentachlorobiphenyl (PCB 126); 2,3,3′,4,4′,5- (PCB 156) and 3,3′,4,4′,5,5′-hexachlorobiphenyl (PCB 169); and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) were purchased from Cambridge Isotope Laboratories (Woburn, Mass.). To determine purity and concentration accuracy of the solutions, aliquots from the isooctane stock solutions were measured by gas chromatography analysis against analytical standards purchased from Cambridge Isotope Laboratories. Analysis of stock PCDD and PCDF solutions was not done. For bioassay procedures, the chemicals were transferred into dimethyl sulfoxide (DMSO) by N_2 evaporation of the isooctane phase.

Culturing techniques

The rainbow trout liver cell line RTL-W1 (Lee et al. 1993) and the rat hepatoma cell line H4IIE (Bradlaw and Casterline 1979) were maintained as previously described by Clemons et al. (1994). For RTL-W1, the normal growth medium was Leibovitz's L–15 (Gibco-BRL) supplemented with 5% fetal bovine serum (FBS; Gibco) and penicillin–streptomycin (100 IU penicillin, 10 μg streptomycin·mL^{-1}; ICN/Flow). For H4IIE, the growth medium was as described in Tillitt et al. (1991). This was Dulbecco's modified Eagle's medium (MEM, Sigma) supplemented with L-glutamine, gentamycin sulphate (50 μg·mL^{-1}), MEM essential amino acids (1.5×), MEM nonessential amino acids (2×), MEM vitamins (1.5×), and 15% FBS. The cell lines were maintained at 22 ± 1°C for RTL-W1 and at 37 ± 1°C in a humidified CO_2 (5%) atmosphere for H4IIE.

Exposure to chemicals

Both cell lines were exposed to the potential EROD inducing agents in 48-well Costar tissue culture plates. Approximately 5×10^5 RTL-W1 and 3×10^5 H4IIE cells were added to each well in 500 μL of normal growth medium. Upon confluency, 2–4 days for RTL-W1 and 1 day for H4IIE, the test agents were added. The chemicals were exposed to the cells such that the DMSO (solvent control) concentration did not exceed 0.5%. Exposure was carried out for 48 h after which the EROD assay was performed.

For RTL-W1, variations on the standard protocol were explored with PCB 169 and TCDD. The cells were grown for up to 14 days in the multiwells prior to the addition of the chemicals. PCB 169 was dissolved in two different carriers: DMSO and isooctane. The test agents were presented to the cells in L–15 supplemented with either 5, 10, 20, or 30% FBS. Exposure to the test chemicals was extended for up to 5 days before EROD activity was assayed.

EROD assay

The assay for EROD activity was performed with intact monolayer cultures in the wells of 48-well culture plates. This method was developed from previous reports on the measurement of EROD activity in living cells (Hammond and Strobel 1992) and within microwell culture plates with a fluorometric plate reader (Donato et al. 1992; Lorenzen and Kennedy 1993). Prior to the EROD assay, exposure to the test agents was terminated by removing the culture medium from the wells and rinsing with 250 μL phosphate buffered saline (PBS, pH 7.5).

Components of the EROD reaction mixture were added to the microwell cultures in two steps. First, all components except the substrate were added. For RTL-W1, the reaction mixture was composed of 0.1 M HEPES buffer (pH 7.8), 7.9 mM $MgCl_2$, and

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0.12 mM NADPH. For H4IIE, the mixture in each well contained 0.1 M HEPES with 29.4 μM dicumarol, 7.9 mM $MgCl_2$, and 0.16 mM NADPH. Dicumarol, which prevents metabolism of the product (resorufin) in rat hepatocytes (Donato et al. 1992), enhanced the EROD activity in H4IIE but had no effect in the current study with RTL-W1 or in a topminnow hepatoma cell line (Hahn et al. 1993). After the above additions, the microwell plate was shaken for 1 min. The reaction was initiated by the addition to each well of 7-ethoxyresorufin (Sigma) to give a final reaction mixture concentration of 0.27 μM for RTL-W1 and 1.63 μM for H4IIE. In both cases, the total volume in the well was 770 μL. The reaction was run for 10 min at 22°C for RTL-W1 and at 37°C for H4IIE. For both cell lines the reaction was linear for at least 15 min.

The product (resorufin) formed and the protein present per culture well were measured with the Cytofluor 2350 (Millipore), a fluorometric multiwell plate reader. For resorufin detection, the excitation and emission wavelengths were set at 530 and 590 nm, respectively. The fluorometric readings were taken immediately after the initiation of the reaction and at the end of the reaction, 10 min later. A standard curve relating fluorometric units to resorufin concentrations was used to convert the observed fluorometric units to picomoles of resorufin formed. After the fluorometric readings for resorufin had been taken, the reaction mixture was aspirated and the cultures prepared for protein measurements. Each well was rinsed with 250 μL PBS and then received 250 μL of distilled H_2O. The plates were placed in a -80°C freezer until the phosphate buffer (HK_2PO_4, 7.49 g·L^{-1}; H_2KPO_4, 0.92 g·L^{-1}; pH 8.0) was added to each well, which now had lysed cell extracts. Fluorescamine (1.08 mM) was used to determine the protein content of the cell extracts as described by Lorenzen and Kennedy (1993). EROD activity was expressed as picomoles resorufin per milligram protein per minute. For both cell lines, the constitutive and TCDD-induced levels of EROD activity measured by this microplate method were similar to the levels we measured previously with cell homogenates (Clemons et al. 1994).

Derivation of mean effective concentration and toxic equivalency factor values

Dose–response data were analyzed by a curve-fitting method that was based on a logit function for continuous response data (Hosmer and Lemeshow 1989) as follows:

$$(1)\quad \text{EROD activity} = \text{background activity} + (\text{EROD}_{max} - \text{background activity})/(1 + e^{\beta-(\gamma\times \ln\text{dose})})$$

where $EROD_{max}$ is the maximal level of EROD induction, background activity is the activity of the solvent control, β is the location parameter for the curve, and γ is the slope parameter for the curve. Through a manipulation of eq. 1, the mean effective concentration (EC50) can be calculated as

$$(2)\quad EC_{50} = e^{\beta/\gamma}$$

Statistics

All statistical tests were performed using SYSTAT software (Wilkinson 1990). Means and standard deviations were calculated within the STATS module. Nonlinear curve fitting of the logit model was done in the NONLIN module. All dose–response curves fit experimental data points with R^2 values ≥ 0.85. A *t*-test for independent means was run within STATS for TEF comparisons between cell lines. Pearson correlations testing for probability of fit and significance were performed in the CORR module for SYSTAT. For all statistical analysis the critical level of significance was $\alpha = 0.05$.

Immunoblotting

For the detection of P4501A1 induction by immunoblotting, RTL-W1 were exposed to the potential inducing agents in confluent cultures in 6-well Costar tissue culture plates. The treatments were DMSO (carrier solvent control), TCDD, and PCB congeners. DMSO was tested at 0.5% (v/v). TCDD was examined over the range of concentrations that elicited a dose–response curve for EROD activity. Individual PCB congeners were tested at a dose at which they induced maximal EROD activity. Each treatment was applied to two wells. The cultures were up to 14 days old upon application of the test agents and the treatments were for 48 h.

Cell extracts or microsomes were prepared from control and treated cultures. Exposures were terminated by aspirating the medium and rinsing each well once with 2 mL of PBS^- (phosphate buffered saline without Ca^{2+} or Mg^{2+}, pH 7.5). The cells were scraped in 2 mL of PBS^-. An additional 2 mL of PBS^- was used to wash a well out and added to the initial 2 mL. Cells from two wells were pooled and centrifuged at 500 × *g* and brought up in 200 μL of PBS^-. Cells were sonicated and frozen at -70°C. In some cases, microsomes were prepared. For this, sonicated extracts were centrifuged for 10 min at 10 000 × *g* and supernatants centrifuged further at 100 000 × *g* for 60 min. The pellet was brought up into 50 μL of PBS and either used fresh or frozen at -70°C. Protein levels were determined using the Bio-Rad microprotein assay method (Bradford 1976). The absorbance was read on a EIA microplate reader using a 590-nm filter and measured against bovine serum albumin (BSA) as a protein standard.

The cell extract was mixed with sample buffer (62.5 mM Tris-HCl, pH 6.8, 10% glycine, 2% (w/v) sodium dodecyl sulfate (SDS), 5% mercaptoethanol, 0.05% (w/v) bromophenol blue), and polypeptides were separated by SDS-polyacrylamide gel electrophoresis (PAGE) with the discontinuous buffer system of Laemmli (1970). The gels were either 10% polyacrylamide or 4–15% gradients of polyacrylamide and run on the mini-Protean II electrophoretic system (Bio-Rad). Equivalent protein (approximately 10 μL) amounts were loaded into each lane. For molecular weight calibrations, the cell extracts were run along with two slightly different sets of molecular weight standards from Bio-Rad: Kaleidoscope prestained standards and Bio-Rad broad-range prestained standards. Both sets contain myosin, β-galactosidase, BSA, carbonic anhydrase, soybean trypsin inhibitor, lysozyme, and aprotinin. In addition, the broad-range set contains ovalbumin. Usually electrophoresis was performed on duplicate gels. After electrophoresis, one gel was stained with Coomassie Brilliant Blue R–250 as described previously (Lee and Bols 1991).

Proteins separated by SDS-PAGE were transferred into nitrocellulose (NTC) for 1 h at 100 V using a Bio-Rad Mini-Transblot apparatus. Following transfer, the NTC was blotted with 3% BSA–PBS (w/v) for 2 h to block nonspecific binding sites. Rabbit anti-trout CYP1A1 (Myers et al. 1993) at a 1:1000 dilution in 1% BSA–PBS was applied to the membrane for a minimum of 2 h. After five washes with PBS, the NTC was incubated with goat anti-rabbit IgG–peroxidase labelled (Sigma) at 1:1000 in 1% BSA–PBS for 1–2 h. Colour development was performed after five washes with PBS using 4-chloro–1-napthol in an H_2O_2-containing solution (Bio-Rad). Development was stopped within 5 min by immersing the NTC in tap water. The membranes were dried between two sheets of filter paper and image analysis was performed using a scanner (Hewlett Packard ScanJet 3p) with an amber acetate filter (Donovan et al. 1994), an image capture and manipulating program (HP Picture Place Software), an image enhancing program (Adobe Photoshop version 3.0), and quantification by ImageQuant software (Molecular Dynamics).

Results

EROD induction

Seven selected PCBs induced EROD activity in H4IIE and yielded sigmoidal dose–response curves of similar shapes (Fig. 1). The maximal activity varied. As a percentage of the TCDD maximum, the maximum was in the 20–30% range for PCBs 105 and 118 and in the 60–90% range for the remaining PCBs. Only PCB 105 at the highest dose showed an indication

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Fig. 1. EROD induction by four non-ortho coplanar PCBs (77, 81, 126, and 169) and three mono-ortho coplanar PCBs (105, 118, and 156) in H4IIE (A) and RTL-W1 (B). For PCBs 77, 81, 118, and 126, 2- or 3-day-old cultures were exposed for 48 h to the PCBs prior to the measurement of EROD activity. The exception was the exposure of RTL-W1 to two mono-ortho coplanar PCBs (105 and 156) and PCB 169, which were added to 7- to 14-day-old cultures for a similar induction period (48 h). EROD activity was expressed as a percentage of the maximal EROD activity (TCDD $EROD_{MAX}$) achieved in 2,3,7,8-TCDD dose–response curves, which were run for each separate experiment. The TCDD $EROD_{MAX}$ ranged between 250 and 600 pmol·mg $protein^{-1}$·min^{-1} for H4IIE and between 80 and 150 pmol·mg $protein^{-1}$·min^{-1} for RTL-W1. Representative dose–response curves are presented. The number of replicates for each congener are listed in Tables 1 and 2.

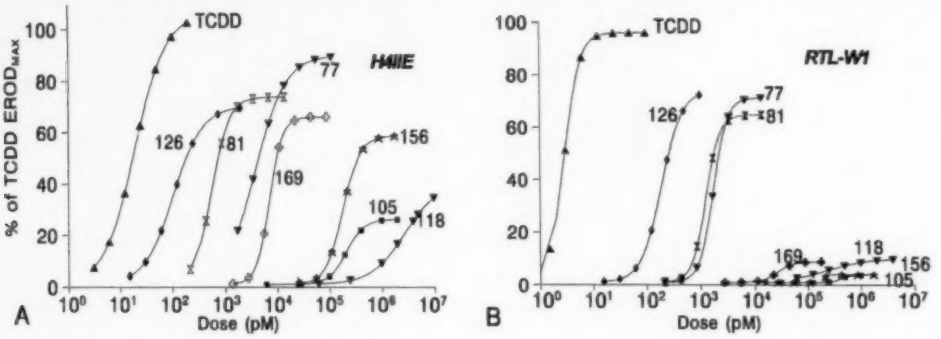
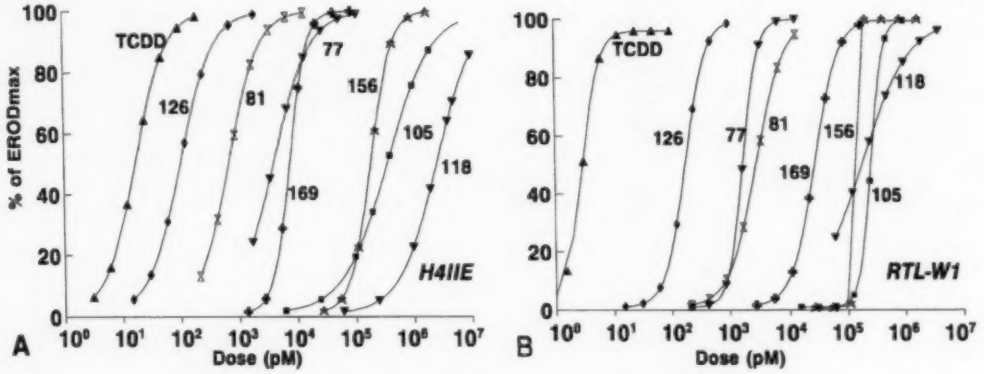


Fig. 2. PCB standardized dose–response curves. EROD activity was expressed as a percentage of the maximal EROD activity for each individual PCB congener to better illustrate the shapes of the dose–response curves in H4IIE (A) and RTL-W1 (B). See Fig.1 caption for details of the experiments.



of cytotoxicity, which was a reduction in the amount of protein per culture well.

EROD activity in RTL-W1 was clearly induced by three non-ortho coplanar PCBs (126, 77, and 81), slightly induced by PCB 118, and induced by the remaining congeners only under exceptional culture conditions (Fig. 2). For PCBs 77, 81, and 126, the maximal induced activity by each congener ranged from 50 to 80% of the TCDD maximum, and the dose–response curves had shapes similar to those of the dose–response curves for TCDD. For PCB 118, the maximal induced activity was less that 10% of the TCDD maximum, and the shape of the dose–response curves was different from those of the TCDD dose–response curves. Under standard exposure and culture conditions, no EROD induction was observed for PCBs 105, 156, and 169, with doses ranging from 0.01 to 3.4 mM. No signs of cytotoxicity were seen with the PCB exposures.

The possibility of detecting induction with different exposure and culture conditions was explored with the inactive congeners. For PCB 169, this included the use of isooctane rather than DMSO as a carrier, varying the FBS concentration during PCB exposure from 5 to 30%, and extending the exposure time up to 5 days. In all cases PCB 169 failed to induce EROD activity. An additional condition was to allow the cells to grow in the multiwell plates for between 7 and 14 days rather than the usual 1–2 days, and then to expose the multiwell cultures to PCB 169 for 48 h. In these extremely confluent RTL-W1 cultures, a low level of EROD activity was induced by this congener and by mono-ortho coplanar PCBs (Fig. 2). For each of these, the maximal activity was always less than 10% of the TCDD maximum (Fig. 2A), and consistent dose–response curves were obtained only for PCB 169. The shapes of the dose–response curves for PCB 169 were similar to those of the curves for TCDD (Fig. 2B).

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Fig. 3. Detection by immunoblotting of P4501A1 induction in RTL-W1. Microsomes were prepared from 9-day-old cultures that had been exposed for 48 h to one of the following: 0.5% DMSO, 48.8 pM 2,3,7,8-TCDD, 53.4 nM PCB 77, 3.9 nM PCB 81, 959 nM PCB 105, 2 μM PCB 118, 967 pM PCB 126, 1.5 μM PCB 156, or 6.3 nM PCB 169. Microsomal polypeptides (10 μg/lane) were separated by electrophoresis on 4–15% gradient SDS-PAGE. The separated polypeptides were immunoblotted with a polyclonal antibody, anti-trout CYP1A1$_{277–294}$/KLH. The arrows identify immunoreactive bands at 58 and 45 kDa and the numbers on the right indicate the sizes of the molecular mass markers in kilodaltons. The 45-kDa band, presumably a degradation product of the 58-kDa band, was only seen under some storage conditions (see text), which were common to all the extracts in this figure.

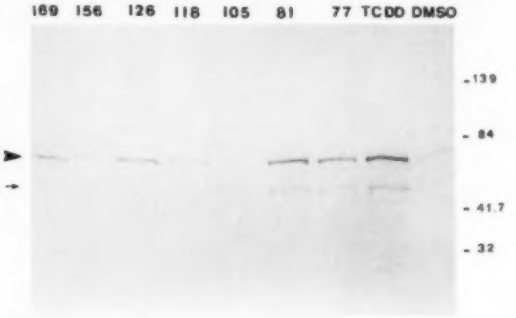
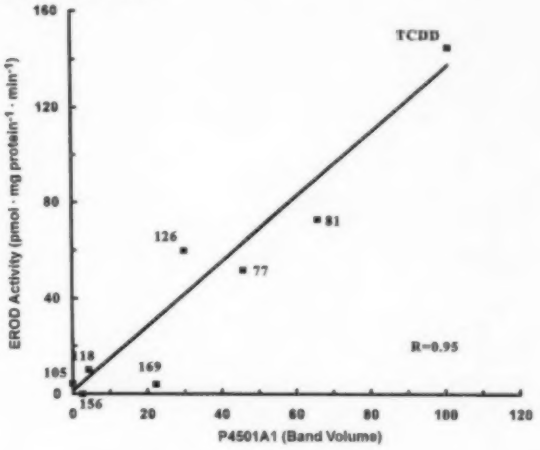


Fig. 4. P4501A1 induction in RTL-W1 as measured by EROD activity and by immunoblotting. The 58-kDa band was quantified as outlined in the Methods and is expressed as P4501A1 (band volume). Treatments, exposure conditions, and imunoblotting were as described in Fig. 3.



P4501A1 induction in RTL-W1

Immunoblotting with anti-trout CYP1A1$_{277–294}$/KLH was optimized on electrophoretically separated polypeptides from RTL-W1 cultures that had been exposed to either 48.8 pM of TCDD or DMSO (carrier solvent control). Electrophoresis of either cell extracts or microsomes shortly after their preparation or after they were stored frozen in PBS revealed a single immunoreactive polypeptide at 58 kDa. Rainbow trout CYP1A1 usually has been reported at approximately 58 kDa (Miranda et al. 1990; Celander and Förlin 1991; Skaare et al. 1991). However, when extracts or microsomes were stored frozen in sample buffer at -70°C for several days prior to electrophoresis, an additional band was immunoreactive. This band was broad, less intensely stained, ran at approximately 45 kDa, and probably can be attributed to degradation under storage conditions. In support of this, a previous thorough characterization of this antibody demonstrated a high level of specificity for CYP1A1 (Myers et al. 1993). In TCDD-treated zebrafish (*Brachydanio rerio*) liver cell cultures, Collodi et al. (1994) detected two immunoreactive bands with a different antibody preparation to rainbow trout P4501A1 and suggested that one could have been a degradation product. For RTL-W1, quantification of immunoblots focused on the 58-kDa band in extracts or microsomes that had been freshly prepared or had been stored in PBS. When quantified, this band was barely detectable in DMSO cultures and increased in cultures exposed to increasing concentrations (1.5, 12.2, and 48.8 pM) of TCDD.

Immunoblotting with anti-trout CYP1A1$_{277–294}$/KLH confirmed that only PCBs 77, 81, and 126 induced CYP 1A1 expression in RTL-W1 under standard exposure conditions. However, when immunoblotting was done on superconfluent cultures that had been exposed to the PCBs at concentrations inducing maximal EROD activity, induction was detected with all PCBs except PCB 105 (Fig. 3). At these doses the level of induction as measured by EROD activity was very similar to the level as measured by immunoblotting (Fig. 4) except for PCB 169. For this congener, EROD activity underestimated the level of induction because activity increased slightly, but band volume increased substantially (Fig. 4). Inasmuch as the concentrations that were tested in Fig. 3 were the ones maximally inducing EROD activity for each PCB and because PCBs 77, 81, and 126 induced similar maximal levels of activity (Fig 1B), the P4501A1 band volumes for these three PCBs would be expected to be similar. The fact that they were different in Fig. 4 appears to reflect variability in the immunoblotting technique, as these differences were not consistently obtained in other immunoblots.

Toxic equivalency factors

We ranked the PCBs for their relative EROD induction potency in the two cell lines by comparing their EC_{50}s (Table 1) and TEFs (Table 2). For RTL-W1, the EC_{50}s and TEFs for PCBs 105, 156, and 169 were derived from superconfluent cultures. PCB 126 was the most potent inducer in both cell lines. The least potent inducer was PCB 118. The TEFs were higher with H4IIE for four of the seven PCBs and were significantly higher for PCBs 126 and 169. Only PCBs 77 and 118 had significantly lower TEFs with H4IIE. Despite these differences, there was a significant linear correlation between the –log TEF values from the two cell lines.

Discussion

The relative ability of the PCBs to induce EROD in RTL-W1 was similar to their ability to induce P4501A upon administration to fish. The three mono-ortho coplanar PCBs were very weak inducers of EROD in vitro with RTL-W1, and similarly

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Table 1. EC_{50} values for selected non- and mono-ortho coplanar PCBs derived for the RTL-W1 and H4IIE cell lines.

		RTL-W1			H4IIE		
			EC_{50} (pM)			EC_{50} (pM)	
Congener	IUPAC	*n*	Mean	SD	*n*	Mean	SD
2,3,7,8-tetraCDD		35	6.13	2.59	43	8.61	3.85
Non-ortho coplanar PCBs							
3,3′,4,4′-tetraCB	77	15	2 239.95	604.53	24	12 007.30	5 182.54
3,4,4′,5-tetraCB	81	13	1 386.17	299.12	10	1 809.43	1 302.92
3,3′,4,4′,5-pentaCB	126	6	229.72	17.34	20	100.60	39.77
3,3′,4,4′,5,5′-hexaCB	169	4	52 723.64	7 047.82	10	6 330.18	1 426.15
Mono-ortho coplanar PCBs							
2,3,3′,4,4′-pentaCB	105	2	170 301.58		3	212 231.79	25 005.41
2,3′,4,4′,5-pentaCB	118	3	345 308.98	168 156.40	10	1 475 058.44	898 950.50
2,3,3′,4,4′,5-hexaCB	156	2	297 863.56		4	133 381.26	62 911.82

Note: *n*, number of trials.

Table 2. Toxic equivalent factors (TEFs) for selected polychlorinated biphenyls derived with the RTL-W1 and H4IIE cell lines.

		RTL-W1			H4IIE			
			TEF			TEF		Proposed
Congener	IUPAC	*n*	Mean	SD	*n*	Mean	SD	TEF
2,3,7,8-tetraCDD			1.0			1.0		1.0
Non-ortho coplanar PCBs								
3,3′,4,4′-tetraCB	77	15	0.003 4*	0.001 8	24	0.000 79*	0.000 30	0.01
3,4,4′,5-tetraCB	81	13	0.006 4	0.001 8	10	0.007 2	0.002 8	
3,3′,4,4′,5-pentaCB	126	6	0.02 3*	0.004 6	20	0.10*	0.045	0.1
3,3′,4,4′,5,5′-hexaCB	169	4	0.000 16*	0.000 048	10	0.001 4*	0.000 37	0.005
Mono-ortho coplanar PCBs								
2,3,3′,4,4′-pentaCB	105	2	0.000 049		3	0.000 025	0.000 008 0	0.001
2,3′,4,4′,5-pentaCB	118	3	0.000 017*	0.000 001 6	10	0.000 010*	0.000 003 5	0.001
2,3,3′,4,4′,5-hexaCB	156	2	0.000 030		4	0.000 052	0.000 016	0.001

Note: Proposed risk assessment TEFs derived from a variety of end points in different species (Safe 1994) are given. *n*, number of assays.
*Statistically significant differences of the mean TEFs between RTL-W1 and H4IIE ($\alpha = 0.05$)

in vivo, two of these have been found to be either inactive or weakly active, whereas the third one, PCB 156, appears not to have been studied. EROD activity was weakly induced after the administration of PCB 118 to rainbow trout (Skaare et al. 1991) and carp (*Cyprinus carpio*) (van der Weiden et al. 1994). In the scup (*Stenotomus chrysops*), neither PCB 105 nor 118 induced P4501A, as judged by EROD activity or by immunoblotting (Gooch et al. 1989). The three non-ortho coplanar PCBs that were clearly active in RTL-W1 have been shown to be active in vivo. The in vivo induction of either AHH, EROD, or immunoreactive P4501A1 has been detected upon the administration to rainbow trout of PCB 77 (Melancon and Lech 1983; Janz and Metcalfe 1991; Vodicnik et al. 1981), PCB 81 (Harris et al. 1994), and PCB 126 (Melancon and Lech 1983; Janz and Metcalfe 1991; Harris et al. 1994). PCBs 77 and 126 also induce EROD activity in carp (Melancon and Lech 1983; van der Weiden et al. 1994). Perhaps the only exception to the good correlation between in vitro and in vivo results was with the non-ortho coplanar congener, PCB 169. Evidence for PCB 169 inducing CYP1A1 expression has been found in sheepshead (*Arochosargus probatocephalus*) (James and Little 1981), scup (Kloepper-Sams et al. 1987), and rainbow trout (Kleinow et al. 1990; Miranda et al. 1990). In rainbow trout this congener induced the activities of EROD (Kleinow et al. 1990) and benzo[*a*]pyrene hydroxylase (Miranda et al. 1990), and immunoblotting confirmed P4501A1 induction. In the case of EROD, the level of activity was considerably less than the level induced by β-naphthoflavone (Kleinow et al. 1990). In RTL-W1 this congener only slightly induced EROD activity.

The TEFs derived with H4IIE in the current study compared favourably with the TEFs derived with H4IIE in previous studies for the same PCBs. The most extensive comparison was with the TEFs derived from the data of Sawyer and Safe (1982) on EROD induction in H4IIE after exposure to the PCBs in the same carrier solvent (DMSO) and for similar times as in the current study. When all of the PCBs in common except PCB 81 were considered, a linear correlation ($R = 0.93$) was obtained from a plot of the -log TEF values from the two studies. Among these six PCBs, differences in TEFs between the two studies were minor except for PCB 105, which had a TEF approximately 20 times smaller in the current study. However, unlike previous data for in vitro induction of P4501A1 by congener 81 in the H4IIE assay (Sawyer and Safe 1982), PCB 81 was found in this study to be a relatively potent inducer of EROD in assays with both H4IIE and trout RTL-W1 cell lines. Further studies should be done to clarify this apparent discrepancy. Generally, few data are available for

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Table 3. Summary of rainbow trout toxic equivalent factors (TEFs) for polychlorinated biphenyls derived from the RTL-W1 cell line, whole fish, and embryos.

		RTL-WI cell line (EROD activity, this study)	Whole fish				Embryos (early life stage mortality)	
			EROD activity	AHH activity				
	IUPAC		(Newsted[a])	Newsted[a]	Janz[b]	Harris[c]	Walker[d]	Zabel[e]
Non-ortho coplanar PCBs								
3,3′,4,4′-tetraCB	77	0.003 4	0.005 4	0.031	0.002		0.0016	
3,4,4′,5-tetraCB	81	0.006 4						0.000 56
3,3′,4,4′,5-pentaCB	126	0.023	0.13	0.054	0.005	0.004	0.005	
3,3′,4,4′,5,5′-hexaCB	169	0.000 16	0.008 8	0.002				0.000 041
Mono-ortho coplanar PCBs								
2,3,3′,4,4′-pentaCB	105	0.000 049	<0.000 05	<0.000 05				No mortality
2,3′,4,4′,5-pentaCB	118	0.000 017	<0.000 06	<0.000 06				No mortality
2,3,3′,4,4′,5-hexaCB	156	0.000 030						No mortality

Note: EROD, AHH, and early life stage mortality were the end points used in the studies. EROD, ethoxyresorufin *o*-deethylase; AHH, aryl hydrocarbon hydroxylase.

[a]Newsted et al. 1995.
[b]Janz and Metcalfe 1991.
[c]Harris et al. 1994.
[d]Walker and Peterson 1991.
[e]Zabel et al. 1995.

this congener (Safe 1990). TEFs for four PCBs (77, 105, 126, and 156) have also been derived from EROD induction in H4IIE after exposure to the PCBs in isooctane rather than DMSO (Tillitt et al. 1991). Except for PCB 156, for which the TEF was the same as in the current study, the TEFs were lower. This difference likely can be attributed to the different carrier solvents. Tillitt et al. (1991) found that isooctane increased the sensitivity of H4IIE to TCDD; DMSO enhanced sensitivity to PCBs 77, 105, and 126; and carrier solvent has little effect on PCB 156.

The potencies of the four non-ortho coplanar PCBs for inducing EROD activity were broadly similar in the rat H4IIE and trout RTL-W1 cell lines but tended to be lower with RTL-W1. When the TEFs derived in this study were used, the TEF for PCB 81 was just slightly lower with RTL-W1, and the large difference noted previously between fish-derived and H4IIE-derived TEFs for this congener (Harris et al. 1994) was not apparent. A significant difference was found with PCB 126, with the RTL-W1 TEF being approximately four times lower. For PCB 77, the TEF derived with RTL-W1 was four times higher. However, preliminary studies suggested that the standard time used in this and previous studies for exposing H4IIE to this congener was too long and that TEFs derived with shorter exposures were up to 10 times higher (Clemons et al. 1993). If this were the case, the RTL-W1 TEF for PCB 77 was a little lower than the H4IIE TEF. The greatest difference was found with PCB 169, which in RTL-W1 induced only a low level of EROD activity and only in older cultures. The RTL-W1 TEF for this congener was approximately nine times lower than the H4IIE TEF. Therefore, the results with the non-ortho coplanar PCBs contrasted with our previous study with polychlorinated dioxins and furans, which generally yielded higher TEFs with RTL-W1 than with H4IIE (Clemons et al. 1994). Together they suggest that differences exist between rainbow trout and rat in the Ah receptor.

The nature of the responses to the mono-ortho coplanar PCBs appeared to differ in RTL-W1 compared with H4IIE, but the TEFs were similar. For these three congeners in RTL-W1, the maximal induced level of EROD activity was consistently very low and profoundly less than the maximal activity achieved by 2,3,7,8-TCDD. With H4IIE, variable maxima after PCB induction have been observed for EROD activity in this study and for AHH and EROD activity in Sawyer and Safe (1982). However, these induction maxima in H4IIE were closer to the TCDD maximum, and in the case of the earlier study, some were higher. In chick hepatocytes, variable maxima between PCB congeners have been observed (Rodman et al. 1989; Yao et al. 1990). Some of this has been attributed to the cytotoxicity at high PCB concentrations (Yao et al. 1990). In the current study, indications of cytotoxicity were seen only with PCB 105 and only with H4IIE; therefore, cytotoxicity was not the cause of the low induction maximum of RTL-W1. Although the low EROD activity made it difficult to estimate dose–response curves for the mono-ortho coplanar PCBs in RTL-W1, they could be estimated consistently for PCB 118. The TEF derived from these curves was 0.000 017, which was almost the same as the H4IIE TEF (0.000 010). In contrast to H4IIE, the dose–response curves for PCB 118 in RTL-W1 appeared to be different from the dose–response curves for TCDD and for the non-ortho coplanar PCBs. It is possible that the mono-ortho PCBs in RTL-W1 have an efficacy or intrinsic activity that is less than that of full agonists. If this is the case, rainbow trout and rat would appear to differ for some ligands in the activity of steps after Ah receptor–ligand binding, such as the Ah receptor dissociating from heat-shock protein (HSP 90), associating with the aryl hydrocarbon nuclear translocator (ARNT), translocating to the nucleus, and binding to dioxin-responsive elements in DNA.

The behaviour of PCB 169 was problematic in RTL-W1. The shape of the dose–response curve for PCB 169 was similar to the dose–response curves for the non-ortho coplanar PCBs. However, like the mono-ortho coplanar PCBs, EROD induction was very low and was only seen when PCB 169 was added a week or so after the RTL-W1 cultures had been initiated.

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Derivation of a TEF was especially complex because of the apparent inhibition of EROD activity in RTL-W1 at high PCB 169 concentrations. Immunoblotting with antiserum to rainbow trout P4501A1 indicated a higher level of protein than EROD activity. In a study in which rainbow trout were injected with PCB 169, high doses appeared to inhibit AHH activity, despite inducing CYP1A1 (Miranda et al. 1990). As pointed out by Hahn et al. (1993), such occurrences could interfere with the derivation of TEFs. Although the development of a sensitive immunoassay for rainbow trout CYP1A1 protein could overcome some problems, the protein level probably would still be too low for PCB 169 and certainly would be for the mono-ortho coplanar PCBs.

Increasing the culture time changed RTL-W1 from being unresponsive to responsive for PCBs 105, 156, and 169. Culture time might have done this by enhancing the detection of P4501A1 induction either in a general way by allowing more cells to be packed into culture wells, or in a specific way by increasing the responsiveness of cells to particular congeners. The latter could have been achieved through the development with longer culture times of a more complex extracellular matrix (ECM). The ECM may influence the induction of phase I xenobiotic metabolizing enzymes in mammalian hepatocytes (Skett 1994) and coordinate hormonal signal transduction (Getzenberg et al. 1990).

The TEFs derived from EROD induction in RTL-W1 compared favourably with TEFs derived from AHH induction in rainbow trout (Janz and Metcalfe 1991; Harris et al. 1994; Newsted et al. 1995) and from early life stage mortality after injection into rainbow trout eggs (Walker and Peterson 1991; Zabel et al. 1995) (Table 3). In all of the rainbow trout systems, TEFs were derived for the non-ortho coplanar PCBs but were difficult to determine for the mono-ortho coplanar PCBs. Collectively, the results suggest that rainbow trout are less sensitive to PCBs than mammals and that the proposed risk assessment TEFs (Safe 1994; listed in Table 2) would appear to overestimate the toxic impact of PCBs on rainbow trout.

In the future, both H4IIE and RTL-W1 should be useful in environmental toxicology. As a bioanalytical tool for detecting PCBs, H4IIE cells appear to be superior to RTL-W1 because they generally yield higher TEFs and clearly respond to the mono-ortho coplanar PCBs. Although still likely to overestimate toxic impact, the TEFs derived with H4IIE more closely resemble the TEFs derived with trout than do the risk assessment TEFs that integrate the results of many different mammalian and avian tests. For PCBs in general, the strengths of the RTL-W1 cells appear to be in quickly identifying the congeners likely to be active in fish and in providing a convenient system for studying potential interactions between congeners, which appear to be different in rat and trout liver cells (J.H. Clemons, L.E.J. Lee, C.R. Myers, D.G. Dixon, and N.C. Bols, unpublished data). The in vitro induction data presented in this study for coplanar PCBs and in a previous study for PCDDs and PCDFs (Clemons et al. 1994) indicate that responses mediated by Ah receptors to halogenated aromatic hydrocarbons are diverse and appear to be species specific.

Acknowledgements

The research was supported by the Natural Sciences and Engineering Research Council of Canada through operating grants and a Great Lakes University Research Fund to N.C.B. and D.G.D. and a scholarship to J.H.C. The work was also supported by the Canadian Network of Toxicology Centres. The gas chromatography quantification and purity analysis of PCB congeners done by Mike van den Heuvel at the University of Waterloo was greatly appreciated.

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Dynamics of a migratory population under different fishing effort allocation schemes in time and space

Dominique Pelletier and Pierre Magal

Abstract: Many exploited fish populations migrate or are confined to some preferential habitats at certain stages of their life cycle. Fishermen may follow some of these spatial and seasonal patterns, in which case the spatio-temporal distribution of fishing effort is closely tied to that of fish abundance. We investigated the dynamics of a population that migrates seasonally in relation to reproduction. Using a two-patch, two-season model, we compared population and catch levels obtained under different spatio-temporal allocations of fishing effort. The model mimics the dynamics of the saithe fishery in the west of Scotland in the 1980s. Results show that better biomass and catch levels could be attained by changing the allocation of fishing effort between seasons and patches, so that the effective age at entry in the fishery is optimized. Similar biomass levels could be obtained through an 80% reduction in total effort without changing its allocation, but catch levels would be much lower. Describing the dynamics of a fishery at a finer spatio-temporal scale thus allows us to explore management measures like openings and closures of zones or seasons.

Résumé : Beaucoup de populations de poissons exploitées migrent ou résident dans des habitats préférentiels à certaines époques de leur cycle vital. Les pêcheurs s'adaptent parfois à ces particularités, et dans ce cas la distribution spatio-temporelle de l'effort de pêche est étroitement liée à celle de l'abondance du poisson. Nous avons étudié ici la dynamique d'une population qui migre chaque année pour aller se reproduire. Grâce à un modèle à deux saisons et deux compartiments, nous comparons les niveaux de population et de captures obtenus pour une gamme d'allocations spatio-temporelles de l'effort de pêche. Le modèle décrit la pêcherie de lieu noir de l'ouest de l'Écosse, telle qu'elle existait à la fin des années 1980. Les résultats montrent que des niveaux de biomasse et de capture plus élevés pourraient être atteints en réallouant un effort total donné entre les compartiments et les saisons, de manière à optimiser l'âge d'entrée dans la pêcherie. Un niveau de biomasse comparable pourrait être obtenu en réduisant l'effort total de 80% sans le réallouer, mais les captures s'en trouveraient nettement diminuées. Cette description spatio-temporelle de la dynamique d'une pêcherie permet d'explorer des mesures de gestion par fermetures ou ouvertures de zones ou de saisons.

Introduction

Most exploited fish populations migrate between some preferential habitats at certain stages of their life cycle. For demersal species living in temperate zones, migration is periodic and closely tied to the seasons. For instance, many demersal species migrate en masse toward spawning areas to reproduce in winter. Thus, reproduction can give rise to high fish concentrations in particular areas. Fishermen often take advantage of this behaviour and target the population at that time.

Therefore, because the spatio-temporal distribution of fishing effort is closely tied to that of fish abundance, ignoring spatial features when estimating fish abundance from commercial catch per unit effort data may lead to substantial biases (Beverton and Holt 1957; Gulland 1977; Pelletier et al. 1993). Also, a population dynamics model that neglects spatial and seasonal features is likely to yield an erroneous stock assessment. Yet there are few attempts to explicitly model the spatial and temporal dynamics of effort and abundance to evaluate alternative management measures (Walters et al. 1993). In most cases, spatial dynamics are not accounted for in operational assessment models, and these models do not allow one to explore management measures like seasonal and spatial openings and (or) closures.

The purpose of this paper is to investigate the dynamics of a population that migrates seasonally to reproduce. We built a model with two seasons and two patches that allows us to consider spatio-temporal management measures, in addition to global measures. This model is used to compare population and catch levels obtained under different allocations of fishing effort between seasons and patches. The model mimics the dynamics of the saithe (*Pollachius virens*) fishery in the west of Scotland that prevailed during the 1980s.

We first describe the saithe population and fishery, then construct a spatio-temporal population model, and compare it with a global (nonspatial) model. Fishing mortalities are modelled as a function of total effort, patch surface areas, catchabilities, and effort allocation parameters. The model is then applied to the data to explore a variety of effort allocation schemes using indicators like population growth rates, biomass, and catch levels. The sensitivity of the results to several parameters is also studied. Finally, we discuss the im-

Received January 11, 1995. Accepted November 20, 1995.
J12713

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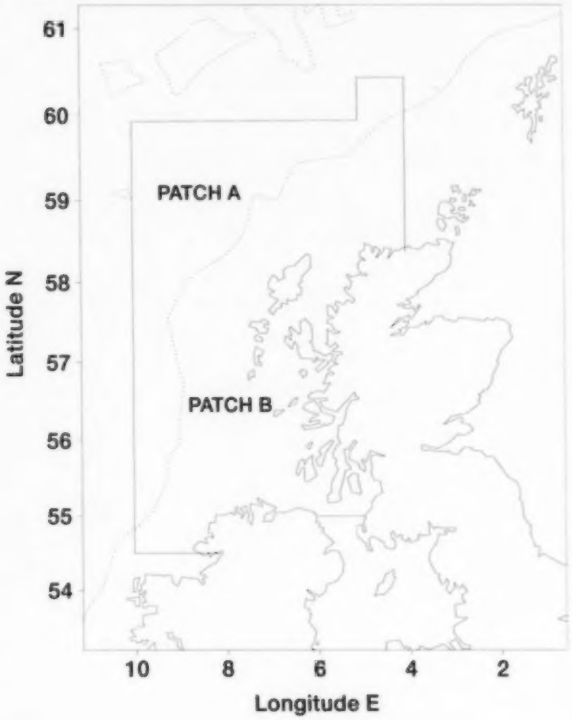
Fig. 1. Management area for the saithe stock in the west of Scotland. The broken line represents the 200-m isobath, i.e., roughly the limit of the continental shelf. Patches A and B are located west and east of this isobath, respectively. The patches correspond to ICES zone VIa. Indeed, the regulatory area (ICES VI zone) for saithe also includes a more western zone (VIb), but saithe catches are negligible there.



plications of the results in terms of spatio-temporal management measures and compare these with global measures like total effort limitation.

The saithe stock in the west of Scotland

Saithe (*Pollachius virens*) exhibits a widespread distribution in the northeast Atlantic, ranging from the west of Scotland to the Arctic Sea. For the purpose of management, five stocks are considered and assessed separately by the International Council for the Exploration of the Sea (ICES): the Arcto-Norwegian, North Sea, Faroe, Icelandic, and west of Scotland stocks. This paper focuses on the latter (Fig. 1), which is the most southern. Mainly exploited by Scottish and French fleets, it has substantially declined in recent years, to the extent that the catch quotas have not been met since 1989 (Anonymous 1992).

Saithe is a highly migratory species; even though stocks are assessed independently, there is evidence of movements between them. Migration studies using tagging experiments have primarily focused on exchanges between the Arcto-Norwegian stock (the largest stock) and the North Sea stock (Jakobsen 1982; Jakobsen and Olsen 1987). The only mark–recapture experiment carried out northwest of the North Sea and west of Scotland indicated a global migration of spawners northeastwards, along the continental slope, toward the North Sea stock (Fontaine et al. 1985). Only occasional migrants could be detected in the opposite direction (from the North Sea toward the west of Scotland). There is apparently no significant exchange with the Icelandic and Faroe stocks. According to current knowledge, the west of Scotland stock can be considered as contributing to the other saithe stocks and there is no evidence for immigration to the area from the other stocks. In this paper, we consider this stock as a biological population, i.e., a self-reproducing group of individuals of the same species.

During the first quarter of the year, spawning adults congregate along the continental slope for reproduction (notice the 200-m isobath in Fig. 1). After spawning, they partially disperse toward more inshore areas. Some migrate northeastwards as indicated earlier. In the western part of the area, the population distribution is limited by depth, which increases sharply beyond the continental slope. Immature fish remain near the coast until they mature, at age 5 approximately. For the purpose of this study, we assume that the area of distribution of the population roughly coincides with the area currently used for assessment and management (Fig. 1).

From January until April, saithe is heavily exploited by large trawlers along the continental slope, where the mature adults congregate to spawn (Fig. 2). We will refer to this fleet as industrial, although these vessels are not factory trawlers. This designation refers rather to their mode of ownership. During this period, the industrial fleet allocates little effort in patch B. During the rest of the year, this fleet's fishing effort is much lower and more evenly distributed over the whole area; often, fishermen do not specifically target saithe, even though they catch some. On average, exploitation by the fleet of large trawlers constitutes up to 80% of the landings, but saithe is also exploited on the continental shelf by a fleet of smaller boats. This effort is allocated closer to the coast; thus, the catch is of more immature fish. Saithe is not necessarily a target species for this fleet. These exploitation features prevailed during the 1980s, but recently the emergence in nearby areas of new resources like deep-water species has modified fishing strategies in the region. We use this example because of the striking relationship between the seasonal distributions of fish and fishing effort.

Population model and assumptions

Biological parameters

The population model is age structured with 11 age groups, the first corresponding to the young-of-the-year, generally called the 0 group. We make the following assumptions: (1) reproduction takes place before fishing, (2) natural mortality is independent of age and equal to 0.2, (3) fishing mortality is age dependent, and (4) knife-edge maturity occurs at age 5.

Assumptions 2 and 4 are not a requirement of the analysis, but rather simplify the equations. Assumptions 1–4 are those on which current ICES assessments are based. Regarding recruitment, there are no research survey indices to study the relation between parental stock and the abundance at age 1.

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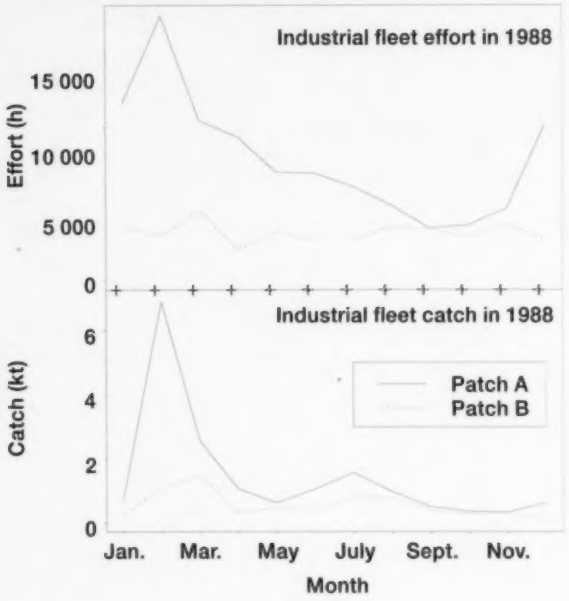
Fig. 2. Allocation of fishing effort and corresponding catch between patches and months for the industrial fleet in 1988.



The time series of historical virtual population analysis (VPA) estimates in Anonymous (1992) is short and offers little insight on this issue. We will therefore consider two alternative hypotheses about reproduction: (*i*) a linear relationship between the abundance at age 1 and the parental stock and (*ii*) a constant abundance at age 1. Assumption *i* corresponds to a situation where there is recruitment overfishing and the stock is low, so that compensatory effects do not affect the survivorship of the young of the year. This assumption could be justified in this application by the intense exploitation level of the stock. Assumption *ii* corresponds to a population with sufficient reproductive capacity, so that the number of young fish surviving the larval phase can be considered as independent of the size of the adult population within certain biomass levels.

These assumptions allow us to build a relatively simple model of the population. At the same time, they are not fundamental for the population dynamics with respect to the question of seasonality and spatial patterns we want to study in this paper. Since there is no information to test hypotheses about reproduction, we will investigate the sensitivity of the results to each of these assumptions.

In addition to the above assumptions, we consider a constant migration rate m for mature age groups migrating from patch A to patch B in spring. We will also explicitly account for the emigration of mature fish toward the northern North Sea to be consistent with the exchanges observed between the different saithe populations (see previous section). The sensitivity of the results to migration parameters will be studied.

Global model

We first consider a model in which patches and seasonality are not taken into account. This model is actually rather close to the current assessment model. Let $N(t)$ be the vector of population size at age at time t. For each age group, fishing mortality at time t and natural mortality are supposed to be independent Poisson processes with respective parameters $F(a,t)$ and M (Seber 1989). The emigration of some mature fish northeastwards outside of the area of interest takes place after reproduction during a short period. A model without seasons cannot capture this feature correctly, so we assume in this model that emigration takes place before fishing. This has consequences for catch rates, but not for survival rates. Let e denote this emigration rate for mature fish, and $p_a(t)$ the survival rate for age a at time t. Under these assumptions, the survival rates are as follows:

$$(1)\quad \begin{cases} p_a(t) = \exp(-(M + F(a,t))), & \forall a = 1,...,4 \\ p_a(t) = (1 - e)\exp(-(M + F(a,t))), & \forall a = 5,...,10 \end{cases}$$

The date at which animals are counted is the 1st day of the year. Every fish changes age group immediately after this date. Therefore, there exists an age group 0 (0 group) comprising the young of the year at time t. Under these conditions, the evolution of stock sizes between t and $t + 1$ is given by

$$N_a(t+1) = p_a(t)N_{a-1}(t), \quad \forall a = 1,...,10$$

Under the assumption of a constant recruitment rate at age 1, the abundance of the young-of-the-year at time $t + 1$ is

$$(2)\quad N_0(t+1) = \sum_{a=5}^{10} b_a N_{a-1}(t)$$

where b_a is the individual fecundity coefficient at age a. Indeed b_a is an effective fecundity coefficient that combines individual fecundity and 0-group survival rate. Note that fish of age 4 at time t contribute to $N_0(t + 1)$ because of the conventional date for changing age groups. Under the alternative assumption of a constant recruitment at age 1, $N_0(t + 1)$ is simply a constant value R. With respect to fish of the last age group, we assumed they still contribute to reproduction for year $t + 1$, i.e., after their 11th birthday; they then die of natural causes, and thus they do not contribute to catches. There are very few fish in this age group, and the hypothesis is not crucial to the model.

By convention, catch $C_a(t)$ represents the catch realized between $t - 1$ and t from fish of age $a - 1$ at $t - 1$, and individuals in the last age group are not totally fished. Consequently, the catch equation is written as

$$C_a(t) = f_a(t)N_{a-1}(t-1), \quad \forall a = 1,...,10$$

where

$$\begin{cases} f_a(t) = \dfrac{F(a,t)}{F(a,t) + M}(1 - \exp(-(F(a,t) + M))), & \forall a = 1,...,4 \\ f_a(t) = \dfrac{F(a,t)}{F(a,t) + M}(1 - e)(1 - \exp(-(F(a,t) + M))), & \forall a = 5,...,10 \end{cases}$$

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Spatio-temporal model

To take into account the spatio-temporal dynamics of the system, we now consider a model with two patches and two seasons. The first season corresponds to the reproductive season, i.e., January to April on average. Patch A corresponds to the spawning area, i.e., the continental shelf between 55 and 60°N latitude (Fig. 1). Patch B corresponds to the inshore zone where immature fish are assumed to reside until age 4. Patch A is substantially smaller than patch B, hence a given number of fish yields a higher density when living in patch A than when living in patch B. The way patches and seasons were precisely determined in practice is described in the section on application.

Reproduction takes place during the first season in patch A exclusively. After spawning, some of the spawners migrate toward patch B, and others definitively migrate northeastwards outside the zone studied. To avoid unnecessary complexity of the model, these migrations are assumed to take place instantaneously at the end of the first season. During the second season, from May to December, every fish remains in the same patch, basically for feeding. At the end of the year, every future spawner migrates toward patch A. At the same time, young-of-the-year arrive in patch B where they stay until maturity. These migrations are also assumed to occur instantaneously.

Fishing activity follows the seasonal and spatial patterns of the population described above. During the first season, we assume that the industrial fleet fishes exclusively for big spawning fish concentrated in patch A. This is a simplification, since data indicate that the industrial fleet also allocates some effort (although little) during the first season in patch B (Fig. 2). After spawner dispersion, this fleet distributes its effort between patches until the end of the year. The inshore fleet is assumed to fish in a uniform way throughout the year in patch B. To construct the spatio-temporal model, it is convenient to consider the dynamics in each patch for each season separately, and then combine them over a 1-year interval (see Appendix for details). Finally, the evolution of stock sizes between t and $t+1$ follows the same equations as in the global model:

$$(4)\quad \begin{cases} N_0(t+1) = \sum_{a=5}^{10} b_a N_{a-1}(t) \text{ or } N_0(t+1) = R \\ N_a(t+1) = p_a(t) N_{a-1}(t), \quad \forall a = 1,\ldots,10 \end{cases}$$

but with survival rates at age a given by

$$(5)\quad \begin{cases} p_a(t) = \exp\left(-(F_1^{\mathrm{B}}(a,t)l_1 + F_2^{\mathrm{B}}(a,t)l_2 + M)\right), \\ \qquad\qquad \forall a = 1,\ldots,4 \\ p_a(t) = (1-m-e)\exp\left(-(F_1^{\mathrm{A}}(a,t)l_1 + F_2^{\mathrm{A}}(a,t)l_2 + M)\right) \\ \qquad + m\exp\left(-(F_1^{\mathrm{A}}(a,t)l_1 + F_2^{\mathrm{B}}(a,t)l_2 + M)\right), \\ \qquad\qquad \forall a = 5,\ldots,10 \end{cases}$$

where F_i^j denotes fishing mortality for season i in patch j, l_i is the length of season i, and m is the migration rate from patch A to patch B at the end of the first season. As explained in the appendix, b_a is the number of young-of-the-year (per spawner capita at age a) surviving after 1 year. Therefore, b_a has exactly the same meaning in the spatio-temporal and in the global model.

The catch equation is the same as eq. 3 with catch rates given by

$$(6)\quad \begin{cases} \forall a = 1,\ldots,4, \\ \quad f_a(t) = \left\{ \dfrac{F_1^{\mathrm{B}}(a,t)}{F_1^{\mathrm{B}}(a,t)+M}\left(1-\exp\left(-(F_1^{\mathrm{B}}(a,t)+M)l_1\right)\right)\right. \\ \qquad + \exp\left(-(F_1^{\mathrm{B}}(a,t)+M)l_1\right)\dfrac{F_2^{\mathrm{B}}(a,t)}{F_2^{\mathrm{B}}(a,t)+M} \\ \qquad \left.\left(1-\exp\left(-(F_2^{\mathrm{B}}(a,t)+M)l_2\right)\right)\right\} \\ \forall a = 5,\ldots,10, \\ \quad f_a(t) = \left\{ \dfrac{F_1^{\mathrm{A}}(a,t)}{F_1^{\mathrm{A}}(a,t)+M}\left(1-\exp\left(-(F_1^{\mathrm{A}}(a,t)+M)l_1\right)\right)\right\} \\ \qquad + \exp\left(-(F_1^{\mathrm{B}}(a,t)+M)l_1\right) \\ \qquad \left\{(1-m-e)\dfrac{F_2^{\mathrm{A}}(a,t)}{F_2^{\mathrm{A}}(a,t)+M}\right. \\ \qquad \left(1-\exp\left(-(F_2^{\mathrm{A}}(a,t)+M)l_2\right)\right) \\ \qquad \left. + m\dfrac{F_2^{\mathrm{B}}(a,t)}{F_2^{\mathrm{B}}(a,t)+M}\left(1-\exp\left(-(F_2^{\mathrm{B}}(a,t)+M)l_2\right)\right)\right\} \end{cases}$$

Evaluation of the dynamics of catch and population

To evaluate the dynamics of both population and catch for a given fishing mortality, we assume that biological parameters and fishing mortality remain unchanged for a number of years, say at least for a generation time, and we compute indicators of population dynamics, catch, and biomass levels in these conditions.

Under the additional assumption of a constant recruitment rate at age 1, the population model can be framed into a Leslie matrix with constant coefficients (Caswell 1989). The evolution of the population over an infinite time horizon is then described by the asymptotic growth rate and the stable age distribution of the matrix. If the growth rate is larger than 1, the population size increases forever, and if it is smaller than 1, the population continuously decreases. The stable age distribution describes the age structure of the population. These measures are independent of initial population values and approximate the population dynamics after a sufficiently long amount of time. Biomass and catch trajectories are also computed to illustrate the evolution of the resource. Because they are computed for a given Leslie matrix, with constant biological parameters and fishing mortalities, these trajectories correspond to equilibrium projections from this standpoint, but catches and biomasses do not stabilize over time, because the population growth rate is never strictly equal to 1. Because of this behaviour, the model is only used for diagnostic purposes on a limited time horizon, and not for prediction purposes. In these conditions, it may be assumed that the parameters of the model remain constant.

Alternatively, under the assumption of constant recruitment, the population reaches equilibrium after a generation, so that both population size and catch become constant and independent of initial population values. In this case, the equilibrium biomass and catch may be computed to provide a diagnostic of the resource. The equilibrium population size is easily expressed as

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$$N^{eq} = R\begin{bmatrix} 1 \\ p_1 \\ p_1p_2 \\ \vdots \\ p_1p_2\cdots p_{10} \end{bmatrix}$$

so that the equilibrium biomass can be written as

$$(7) \quad B^{eq} = \sum_{a=0}^{10} w_a N_a^{eq}$$

From eq. 3, we get the equilibrium catch:

$$(8) \quad C^{eq} = \sum_{a=0}^{10} w_a f_a N_a^{eq}$$

C^{eq}/R corresponds to an equilibrium yield per recruit.

Modelling fishing mortality and fishing effort allocation

The purpose of the paper is to study the consequences on population and catch of the intensive exploitation of fish concentrations during one season. More generally, we want to explore the population and catch levels obtained under different allocations of fishing effort between patches and seasons. Therefore, we need to define the relationship between fishing mortality and a given fishing effort allocation. Let F denote the vector of instantaneous fishing mortality rates in the global model, each rate being expressed in years^{-1}. In many assessment models, it is assumed that fishing mortality is proportional to fishing effort E:

$$(9) \quad F = qE$$

where q is a constant vector of individual catchability coefficients q_a. Considering the fishing mortality as a Poisson process, q_a represents the probability that an individual fish of age a present in the area of distribution will be caught by a unit of effort during the time interval chosen. Fish of a given age are thus assumed to be equally vulnerable to fishing. However, the assumption of a constant q may be invalidated in several instances such as density-dependent catchability, spatial heterogeneities, and technological developments in the fleet. In this study, we focus on spatial heterogeneities at the scale of the seasonal changes in fish distribution.

Introducing the spatio-temporal model allows us to account for the differences in both patch sizes and season lengths. Because patch B is substantially larger than patch A, individual catchability is higher in the latter, all other things being equal. In these conditions, catchabilities in patches A and B, say q^A and q^B, should be related to overall catchability q through the ratio of patch surface area (where a given unit of effort is applied) to the total area of fish distribution:

$$\begin{cases} q^A = ((s_A + s_B)/s_A)q \\ q^B = ((s_A + s_B)/s_B)q \end{cases}$$

To explore the effects of patch sizes, catchability per unit of surface is assumed to be constant throughout the area of distribution of the population. Thus, the assumption of eq. 9 does not hold at the global scale but is used within a patch for a given season. This means that fishing gears have a constant efficiency whatever the patch and the fleet, and that fish are randomly distributed within each patch. With this model, a given fishing effort E generates a higher mortality in the smallest patch (where fish congregate for reproduction).

In addition, the two seasons have different lengths, the first one comprising only one third of the year. Fishing effort is measured in numbers of trawled hours (and not in numbers of boats, for instance) per time interval. All other things being equal, a given number of fishing hours must imply the same relative decrease in stock sizes at the end of the season, regardless of season length. Therefore, it is necessary to rescale fishing efforts with respect to the length of each season when expressing fishing mortalities.

Let E_1^A, E_2^A, E_1^B, and E_2^B be the fishing efforts allocated in each patch, A and B, during each season, 1 and 2. Let us denote s_j the surface areas of patch j, and l_i the length of season i. Fishing mortalities are finally formulated as

$$(10) \quad \begin{cases} F_1^A = q^A E_1^A / l_1 = (s_A + s_B)/s_A q E_1^A / l_1 \\ F_2^A = q^A E_2^A / l_2 = (s_A + s_B)/s_A q E_2^A / l_2 \\ F_1^B = q^B E_1^B / l_1 = (s_A + s_B)/s_B q E_1^B / l_1 \\ F_2^B = q^B E_2^B / l_2 = (s_A + s_B)/s_B q E_2^B / l_2 \end{cases}$$

We will consider a constant total fishing effort E for the year over the whole area and explore a range of possible allocations of E over patches and seasons. We make two additional assumptions to obtain a simple model of effort allocation: (*i*) the inshore fleet generates a constant mortality rate all year long in patch B (recall that this fleet fishes exclusively in patch B) and (*ii*) during the second season, the industrial fleet generates the same mortality rate in both patches. These assumptions imply rescaling corresponding fishing efforts with respect to season length and patch surfaces, respectively. Alternatively, we may have considered equal nominal efforts rather than equal mortality rates in the previous assumptions. The assumption of equal mortality rates facilitates the comparison with the global model because from the population standpoint, the spatial heterogeneity then strictly pertains to the concentration of fishing effort during the first season in patch A.

Under assumptions *i* and *ii*, effort allocation over patches and seasons is parameterized as follows :

$$(11) \quad \begin{cases} E_1^A = (1-\beta)\,\alpha E \\ E_1^B = \beta l_1 E \\ E_2^A = (1-\beta)(1-\alpha)\dfrac{s_A}{s_A + s_B} E \\ E_2^B = \beta l_2 E + (1-\beta)(1-\alpha)\dfrac{s_B}{s_A + s_B} E \end{cases}$$

with $E_1^A + E_1^B + E_2^A + E_2^B = E$. Parameters β and α quantify the total effort corresponding to the inshore fleet and the fraction of the industrial fleet's effort allocated in patch A during the first season, respectively. In terms of survival rates, the global model is equivalent to $\alpha = 0$ and $\beta = 0$. This parameterization does not capture all possible allocation schemes of fishing effort between seasons and patches, but it accounts for the existence of the two fleets: a highly seasonal industrial fleet and a nonseasonal inshore fleet. Because total effort E is constant, fishing effort allocation is simply determined by α and β.

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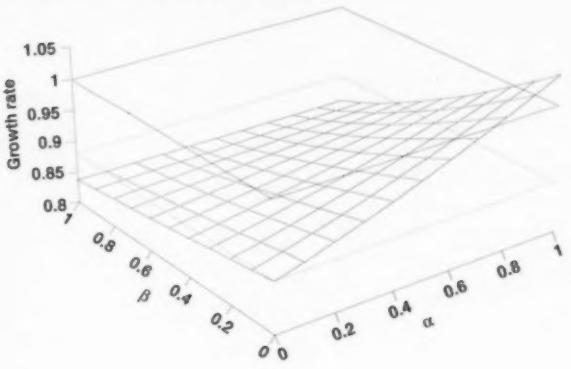
Fig. 3. Population growth rate as a function of β, the importance of the nonseasonal inshore fleet, and α, the concentration of fishing effort on the continental shelf in winter. The empty planes correspond to the growth rate in the global model (broken line) and to a growth rate of 1 (solid line).



Application and results

To compare population and catch obtained under different fishing effort allocations, we computed the indicators of population and catch levels defined in the subsection entitled Evaluation of the dynamics of catch and population. We studied how migration rate m and effort allocation parameters influence the dynamics of both population and catch. We also examined the sensitivity of the results to several parameters and assumptions. We successively present results in the case of a linear stock-recruitment relationship, i.e., a constant recruitment rate at age 1 (termed the constant b case) and in the case of a constant recruitment at age 1 (termed the constant R case).

The dynamics of both spatial and global models are studied in the case of the saithe stock, whose biological characteristics were described in a previous section. Natural mortality and weights at age are taken from Anonymous (1992). Regarding recruitment, there is unfortunately no information available from surveys or previous studies about the biology of this population. Therefore, we consider the two alternative assumptions mentioned in the previous paragraph. In the constant b case, there is not enough information to estimate age-dependent fecundity coefficients without additional arbitrary hypotheses; therefore, we assume $b_a = b$ and crudely compute b from VPA stock size estimates as the ratio of the mean number of fish of age 1 to the mean number of mature fish in the previous year. This is consistent with the definition of b_a in eq. 2. In the constant R case, recruitment R is estimated as the historical mean value of VPA stock size estimates at age 1 in Anonymous (1992). Initial population sizes used for computing trajectories correspond to 1988 VPA estimates in Anonymous (1992). As for migration rates, there is no information nor are there experimental results concerning the value of the between-patch migration rate m, which we assume to be unknown. Conversely, the emigration rate e is set equal to 0.4 following Fontaine et al. (1985). Since no uncertainty was attached to this estimation in the latter study, we will study the sensitivity of the results to this parameter.

Definition of patches

To define precisely the extent of each patch, logbook data for the industrial fleet were used. The information reported in the logbooks consists of catch, fishing effort (in numbers of trawled hours), and fishing location for each vessel on a per trip basis. Fishing location is defined by a statistical rectangle that is the finest subdivision of ICES regulatory areas. The dimensions of a statistical rectangle are 30° latitude and 1° longitude, which in this zone roughly corresponds to a 30 nautical mile square. Multivariate data analyses were carried out with the logbook data on a monthly basis and for each statistical rectangle. This way, statistical rectangles could be grouped into two patches and two seasons according to their profiles of effort and catch (see for instance Fig. 2). Rectangles that represented negligible catch and effort levels were excluded from the application, particularly west of the continental slope.

Exploitation and effort allocation parameters

Exploitation characteristics are taken from Anonymous (1992). Since the early 1990s, exploitation by the industrial fleet has undergone thorough changes, in particular with the emergence of fishing for deep resources. Therefore, our study is based on the 1988 patterns of fishing mortality and fishing effort. A vector of catchabilities at age q is simply derived as $q = F/E$ where F is the fishing mortality vector for 1988 estimated from the VPA model used for the assessment in 1992 and E is the total effort of the industrial fleet in trawled hours for 1988. Parameter α can be computed from the industrial fleet effort data as approximately 0.2. The computation of β requires effort data for the inshore fleet. However, these data were not considered here because the effort measures of the industrial and inshore fleets are not comparable: neither the boat sizes, nor the gears used (different trawl types), nor the fishing techniques, nor the fishermen tactics (particularly the degree of targetting for saithe) are similar. No information is available to intercalibrate effort data by rescaling catchabilities. As the inshore fleet catch amounts to about 10% of total catch, it is more reasonable to neglect its effort when computing catchabilities, rather than combine incompatible data. This will probably introduce a slight error in the computation of catch and biomass levels in the real case situation. However, global and spatial models will be affected in the same way, and this approximation will be of little consequence when comparing different spatio-temporal allocations of fishing effort. The impact of the coastal fishing activity will rather be investigated through the parameter β (see eq. 11). Since the inshore fleet catch amounts to only 10% of the total catch, β is likely to be small, probably between 0.1 and 0.3.

Population dynamics

The first (and unexpected) result is the general insensitivity of the dynamics to the value of the migration rate m whatever the assumption about reproduction. Therefore, only results for m = 0.3 will be presented.

In the constant b case, the population dynamics is illustrated by the population growth rate, the biomass trajectories, and the stable age distribution. Under the effort allocation prevailing in 1988 (termed actual effort allocation), i.e., $\alpha = 0.2$ and plausibly $0.1 < \beta < 0.3$, the population growth rates in the spatial model and in the global model are very similar, around 0.89.

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Fig. 4. Evolution of the biomasses over a 20-year period for several values of α and β: α = 1 and β =0.2, α = 1 and β = 0.4, α = 0.2 and β = 0.2, and α = 0.2 and β = 0.4. The trajectories for the global model are very close to those of the last case (α = 0.2 and β = 0.4).

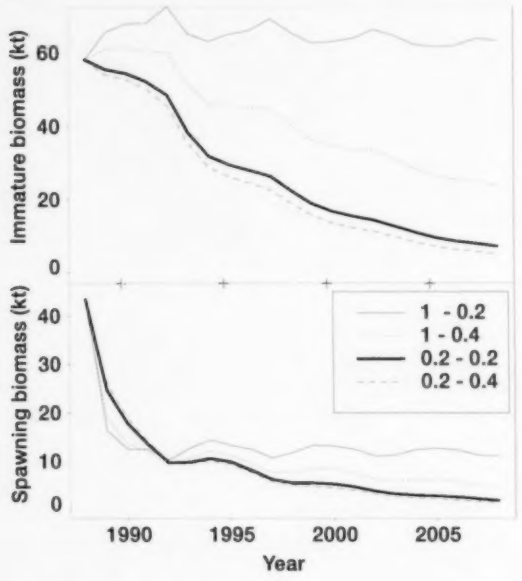


Table 1. Immature biomass in tonnes after a generation time for several values of β and α (constant *b* case).

	α		
β	0	0.2	1
0	16 694	22 880	111 465[a]
0.2	14 887	18 887[b]	62 679
0.4	13 331	15 743	36 313
1	9 869	9 869	9 869

Note: The corresponding biomass in the global model is 16 694 t.
[a]The effort allocation that maximizes both population and catch levels.
[b]The actual effort allocation.

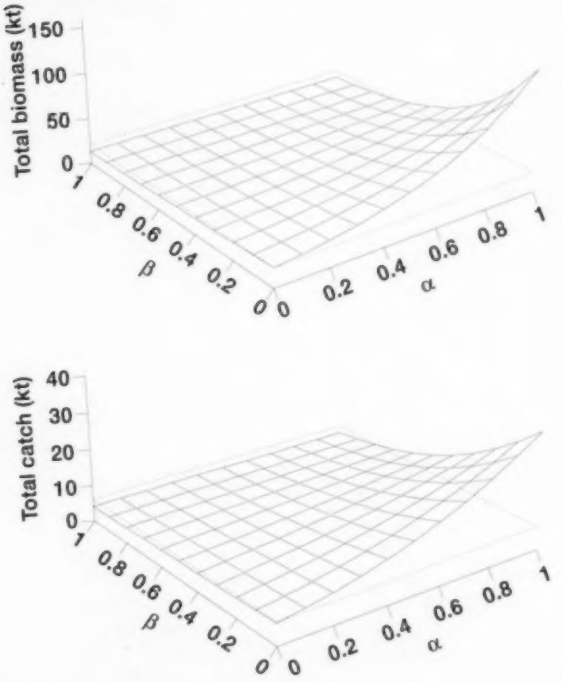
Table 2. Mature biomass in tonnes after a generation time for several values of β and α (constant *b* case).

	α		
β	0	0.2	1
0	5277	6322	21 134[a]
0.2	4860	5462[b]	13 316
0.4	4515	4803	8 584
1	3876	3876	3 876

Note: The corresponding biomass in the global model is 5277 t.
[a]The effort allocation that maximizes both population and catch levels.
[b]The actual effort allocation.

From this standpoint, the population is overexploited. The population growth rate in the spatio-temporal model becomes

Fig. 5. Total biomass and total catch as a function of β and α after a generation time (constant *b* case). The broken line corresponds to the global model.



larger than in the global model roughly when α is larger than β (Fig. 3). Under these conditions, the spatial growth rate becomes more sensitive to changes in α and β. Logically, the influence of α is mainly apparent at small β values, i.e., when the industrial fleet effort constitutes the major component of exploitation. The largest growth rates are observed for simultaneously high α and low β values. They become even larger than 1 when $\alpha \geq 0.8$ and $\beta \leq 0.2$. These results indicate that for a constant total effort, when fishing concentrates even more on spawners (α increases), the population growth rate does not decrease. In contrast, allocating more effort to catching immature fish (by increasing β) drastically affects the whole population. In fact, the population growth rate is driven primarily by the survival rate of the immature fish. Note that the intensive exploitation of the spawners takes place after reproduction (the consequences of this assumption will be evaluated later).

In general, the absolute magnitude of the growth rate does not vary much according to the parameters considered, but a small variation in the growth rate may induce large changes in population sizes as exemplified by biomass trajectories (Fig. 4). Recall that such trajectories are not predictions of future biomasses, but only represent equilibrium projections, in that all parameters remain constant over the projection horizon. Monotonically decreasing trajectories correspond to growth rates smaller than 1. Steadily increasing biomasses can be observed for α = 1 and $\beta \leq 0.2$ (not represented here). Note that the general decrease in spawning biomass in the first years is sharp only because of the initial stock sizes.

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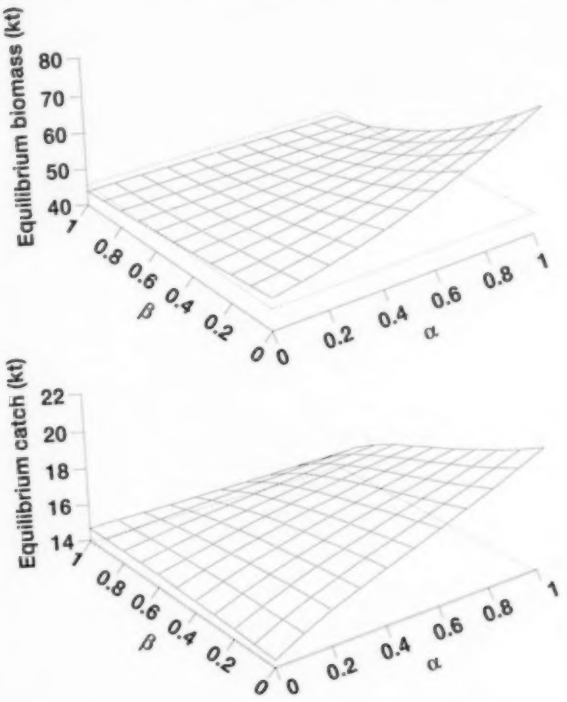
Table 3. Age distributions obtained from the spatial model for two different effort allocations (β = 0.2 in every case).

Effort allocation (α)	Reproduction	Age group (years)						
		0	1	2	3	4	5	6–10
0.2	Constant *b*	0.284	0.261	0.203	0.125	0.071	0.033	≤0.015
0.2	Constant *R*	0.323	0.264	0.191	0.115	0.065	0.027	≤0.010
1	Constant *b*	0.295	0.230	0.180	0.141	0.110	0.035	≤0.009
1	Constant *R*	0.293	0.240	0.187	0.136	0.096	0.034	≤0.010

Note: The age distributions correspond to equilibrium in the constant *R* case, and to the stable age distributions in the constant *b* case.

Fig. 6. Equilibrium biomass and catch as a function of β and α (constant *R* case). The broken line corresponds to the global model. Note the difference between scales when comparing with Fig. 5.



Examining biomass levels after a generation time stresses the influence of β and α (Fig. 5, Tables 1 and 2). Biomass variations may of course be very high when comparing extreme values of the parameters, but it appears that slightly modifying the values of α and β from the actual effort allocation may produce significant changes in biomass values. Immature biomass is much more sensitive to changes in effort allocation than mature biomass. Again, results show that what matters to the population is not fishing on spawners, but fishing pressure on immature fish. Thus, when all the fishing pressure is concentrated on mature fish during the first season (α = 1 and β = 0), mature biomass is maximal, and after a generation time it is 2–5 times as high as under any other allocation scheme considered (Table 2). Under these conditions immature biomass is maximal too (Table 1). Therefore, it seems that in the long term, the persistence of the population is not affected by a very high fishing pressure on spawners. Biomasses relative to the actual situation (α = 0.2 and 0.1 < β < 0.3) are substantially larger in the spatial model than in the global model, although corresponding population growth rates were found to be similar.

Table 4. Immature catch in tonnes after a generation time for several values of β and *a* (constant *b* case).

β	α		
	0	0.2	1
0	4911	5467	0[a]
0.2	4688	5121[b]	5489
0.4	5974	5607	6142
1	3901	3901	3901

Note: The corresponding catch in the global model is 5094 t.
[a]The effort allocation that maximizes both population and catch levels.
[b]The actual effort allocation.

Table 5. Mature catch in tonnes after a generation time for several values of β and α (constant *b* case).

β	α		
	0	0.2	1
0	1227	3289	32 015[a]
0.2	1010	2414[b]	16 432
0.4	821	1734	8 319
1	380	380	380

Note: The corresponding catch in the global model is 1272 t.
[a]The effort allocation that maximizes both population and catch levels.
[b]The actual effort allocation.

In the constant *R* case, the equilibrium biomass (reached after a generation) shows qualitatively similar variations with fishing effort allocation, being maximal for α = 1 and β = 0 (Fig. 6). However, on the whole it is less sensitive to α and β.

Note finally that whatever the assumption about recruitment, the age structures of the population are similar in most cases because the stable age distribution is not sensitive to parameters α, β, and *m* (Table 3). The spatial and global models yield similar results.

Catch dynamics

Similarly to the situation for biomasses, catch is not sensitive to the migration rate *m*, and mainly varies according to α and β (Figs. 5 and 6). Whatever the assumption about reproduction, total catch after a generation is maximal for β = 0 and α = 1, but it is much less sensitive to these parameters in the

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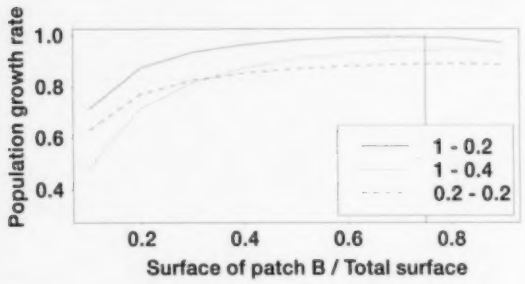
Fig. 7. Sensitivity of the asymptotic growth rate to the relative surfaces of the patches for several values of α and β: $\alpha = 1$ and $\beta = 0.2$, $\alpha = 1$ and $\beta = 0.4$, $\alpha = 0.2$ and $\beta = 0.2$, and $\alpha = 0.2$ and $\beta = 0.4$. The vertical line indicates the real value ($s_B/(s_A + s_B) = 0.75$).



constant R case than in the constant b case. In the latter case, it may vary from 4281 t for $\beta = 1$ (independently of the value of α) to 32 015 t for $\beta = 0$ and $\alpha = 1$ (Tables 4 and 5). Moving from the actual effort allocation (7535 t) to the one that maximizes total catch (referred to hereafter as best effort allocation) would expand total catch fourfold. Recall that these values do not stabilize over years in the constant b case. Similarly to the situation for biomasses, catch trajectories (results not shown) emphasize descending or increasing trends in the case where population growth rates are smaller or greater than 1, respectively. In the constant R case, total catch is at equilibrium after a generation and roughly varies from 14 000 to 18 000 t. In this case, moving from the actual effort allocation to the best one would raise total catch from 14 588 to 17 721 t, i.e., an increase of only 21%. Considering mature and immature catches separately, we show that in any case, catch variations above all reflect changes in mature catch levels. As was found for biomass, a maximal catch is obtained when fishing effort exclusively targets mature fish during the first season.

Sensitivity analyses

At this point, it is necessary to investigate carefully the importance of some parameters and assumptions to (*i*) thoroughly understand why catch and population dynamics vary so much with the spatio-temporal allocation of effort and (*ii*) ensure that our results are not too sensitive to parameters that were crudely estimated or may undergo random fluctuations, or to assumptions that may prove difficult to check. Several parameters may a priori be important in investigating effort allocation between patches and seasons: the between-patch migration rate m, the emigration rate e, the ratio of the patch sizes, and season lengths. Migration rate m has proved to be a minor parameter throughout the analysis, and therefore will not be studied further. Note also that our results do not depend upon the relative lengths of the seasons because fishing effort is measured in hours per season. Hence, instantaneous fishing mortalities depend upon the lengths of the seasons, but not the mortality rates integrated over a given season. It would be different if effort were measured in number of boats (or gears) used during a season.

We considered the sensitivity of the results to the emigration parameter e. Unreported results show that the population growth rate is systematically affected in the same way regardless of effort allocation. Thus, the value of the emigration rate e does not generate qualitative changes in the results.

We then examined the influence of relative patch size in the constant b case. Population growth rates were computed over a range of values of $s_B/(s_A + s_B)$ (Fig. 7). Recall that as $s_B/(s_A + s_B)$ increases, the fishing mortality induced by a given effort decreases if this effort is applied in patch B and increases if it is applied in patch A. The values explored range between 0.1 and 0.9 to avoid unreasonably high fishing mortalities by reducing patch sizes too much. Results show that whatever the allocation of fishing effort, increasing F on spawners is not a problem: if patch A were even smaller, growth rates would not be much affected. In contrast, it would be harmful to the population to increase F on immature fish (for instance if patch B were smaller), since the growth rate would quickly drop below 0.8. This decrease would be mitigated in the case of moderate fishing pressure on immature fish, approximately when $\beta \leq 0.2$ and $\alpha \geq 0.8$ (cf. thin solid line in Fig. 7). In these cases, patch B should theoretically be smaller than patch A (i.e., $s_B/(s_A + s_B) < 0.5$) for the growth rate to drop below 0.9. Relative patch size is thus a sensitive parameter, because it is important for the relationship between fishing mortality and fishing effort. This result may be helpful if we want to discuss our results from a more general standpoint (see next section).

We also checked the consequences of assumption 1 (reproduction takes place before fishing). Reproduction takes place during the first quarter and there certainly exists some overlap in time between reproduction and fishing. To check the consequences of this possible overlap for our analysis, we assumed that the date at which reproduction takes place is uniformly distributed during the first season. Subsequent growth rates are only slightly modified (results not shown), and the relative performances of different effort allocation schemes are not qualitatively affected.

Finally, we checked the sensitivity of our results to recruitment parameters and to assumptions regarding the stock-recruitment relationship, assumptions that are likely decisive for the results.

The effective fecundity coefficient b, or alternatively the recruitment R, could only be crudely estimated. Also, these parameters may fluctuate randomly in relation to environmental conditions. We investigated these effects in the constant b case by carrying out 200 Monte-Carlo simulations, assuming a lognormal distribution for b with an arbitrary coefficient of variation (CV) of 50%. We found that the sensitivity of the population growth rate to random fluctuations in b is low and almost independent of α and β with a CV of approximately 7%. Logically, biomass and catch projections are much more sensitive, particularly as the time horizon for projections increases. After a generation time, biomass and catch CVs are approximately 94 and 89% under the actual allocation, versus 100 and 86% when $\alpha = 1$ and $\beta = 0$. Unlike catches, biomasses are slightly more sensitive for allocation schemes that yield the highest population growth rates. In the constant R case, C^{eq} and B^{eq} are proportional to R (see eqs. 7 and 8). Therefore, if R is a random variable with a given CV, C^{eq} and B^{eq} have the same resulting CV, which determines their sensitivity to R.

In the application, we considered an age-independent b because of a lack of information for estimating this parameter. We investigated the sensitivity of the results to a possible age

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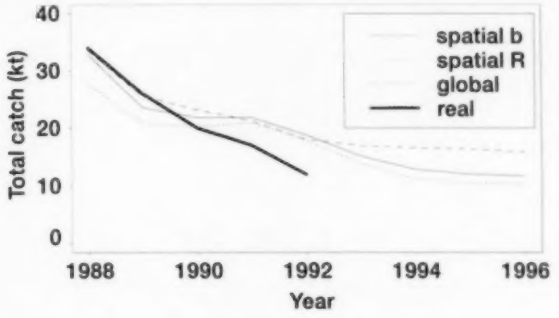
Fig. 8. Comparison between estimated real catch (real) and catch obtained from the models: global model (global), and spatio-temporal model for $\alpha = 0.2$ and $\beta = 0.2$ under the constant b assumption (spatial b) and under the constant R assumption (spatial R).



dependence of b considering that b_a is likely to be linked to individual weight at age a. There are different ways to achieve this and we explored several of them, so we only report qualitative results. Introducing age dependence for b amounts to increasing it for old age groups and decreasing it for young ones. Since the latter are more numerous in the population, this has the same effect as decreasing an age-independent b. Unreported results show that better allocation schemes, which used to have the highest population growth rates (α close to 1 and β close to 0), are more affected by a change in b than the other allocation schemes. When b diminishes, or equivalently when it is age dependent, population growth rates corresponding to better effort allocation schemes become closer to those of the other schemes, but still remain higher in any case.

In the analysis, we investigated two contrasting stock-recruitment relationships, a linear one and a constant recruitment. We also examined the dynamics of the model under a compensatory stock-recruitment relationship, e.g., a Ricker relationship (results not shown). As might be expected, the addition of a compensatory effect reduces the biomass and catch levels that can be observed for better allocation schemes. Biomass and catch trajectories are similar to those observed with a low constant coefficient b.

We conclude from these additional computations that our results about the relative performances of a range of fishing effort allocations are not qualitatively dependent upon the assumptions about reproduction, nor upon the value of the emigration rate or the fecundity coefficient. However, absolute levels of catch and biomass may be affected. In particular, the biomasses and catches presented above in the constant b case for better allocation schemes may be overly optimistic if (*i*) the overall reproduction level of the population is overestimated in the model, (*ii*) b depends upon age in reality, or (*iii*) there is a compensatory stock-recruitment relationship.

Discussion

Although this was not the main objective of the paper, available information allowed us to provide a diagnostic about the saithe stock in the west of Scotland under the exploitation regime prevailing during the 1980s. Under the constant b assumption, the level of fishing effort and its allocation between seasons and patches in 1988 corresponds to a population growth rate of 0.89; therefore, the population should become extinct over an infinite time horizon if biological parameters and effort features remain unchanged. The population growth rate in the global model is close to that in the spatial model. Under the constant R assumption, the population cannot become extinct; the catch model is then close to an equilibrium yield per recruit model and only allows a possible diagnostic of growth overfishing (see below).

Catch projections are obtained from constant exploitation features; they may be compared on the short term with catches in 1988–1992. Both exhibit a similar descending trend (Fig. 8). The deviation between catches obtained from the model and real catches increases from 1991 onwards, probably in relation to changes in fishing strategies. As mentioned earlier, fishermen started to target deep resources after 1990.

The performances of the spatio-temporal model and the global model may also be compared. In general, the growth rate is higher in the first case. The difference in growth rates between the two models increases as the seasonality and heterogeneity of the exploitation increases, i.e., as the nonseasonal fleet becomes marginal (β diminishes) and the industrial fleet concentrates more on spawner aggregates during winter (α increases). This is because in the spatial model, the relationship between fishing effort and fishing mortality is described more accurately. In particular, because this model accounts for the fact that the industrial fleet mainly targets spawners during the first season, corresponding fishing effort does not induce mortality on immature fish.

Exploration of alternative allocation schemes for fishing effort

The spatio-temporal model presented above was mainly helpful in investigating catch and population dynamics under a range of fishing effort allocation schemes over patches and seasons, although the parameterization used does not capture all possible schemes. We showed that concentrating effort on spawners should not be harmful to the population, even if it were to occur within a small area (thus inducing very high fishing mortalities). In contrast, the population level is substantially affected by the mortality rate of immature age groups. This is all the more striking since patch B (where immature saithe live) is three times as large as patch A, so that a given fishing effort yields a mortality rate in patch B approximately one third of what it would be in patch A.

Under this model, the best allocation of fishing effort, i.e., the one that maximizes both population and catch levels, would be to fully concentrate on mature fish during the spawning season. This is true regardless of which assumption about reproduction is chosen among those considered in the analysis. Under the best allocation, immature age groups are simply not exploited. The age of recruitment to the fishery is thus increased from 2 to 5 years, which prevents recruitment overfishing in the constant b case. In the constant recruitment case, our model is a spatio-temporal version of the classical yield per recruit model (Thompson and Bell 1934; Beverton and Holt 1957). In this case, increasing the age of recruitment to the fishery prevents growth overfishing. Beverton and Holt (1957) also showed that for a fast growing species intensely exploited, it is desirable to delay the age of recruitment, so as to take full advantage of the fast growth during the first years.

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Fig. 9. Biomass and catch trajectories obtained when introducing the best allocation scheme ($\alpha = 0$ and $\beta = 1$) after 6, 10, or 14 years (constant *b* case). The thick solid line corresponds to a status quo situation ($\alpha = 0.2$ and $\beta = 0.2$). Dots correspond to introducing the best allocation scheme in the 1st year.

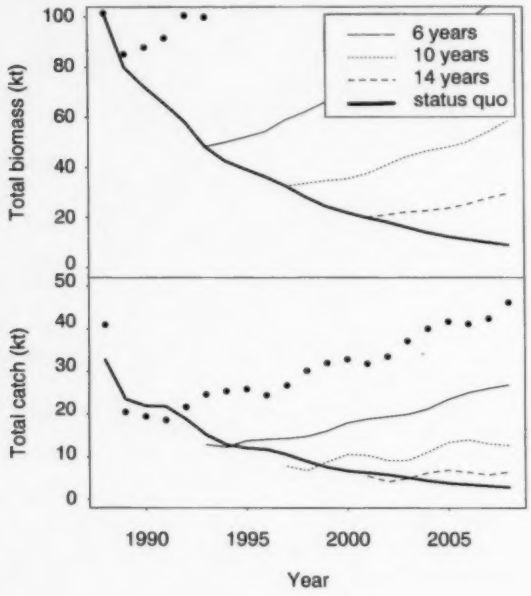
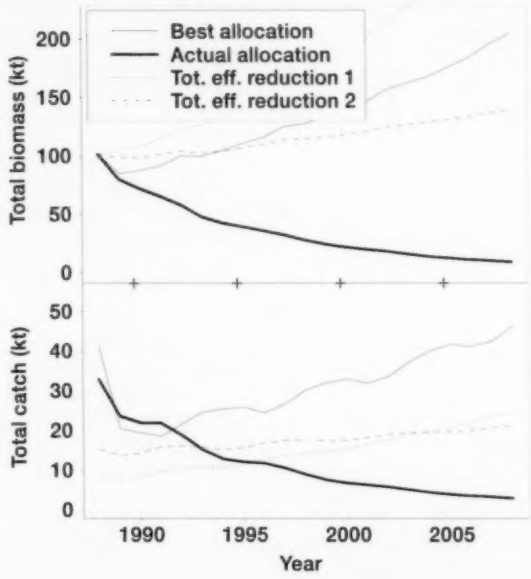


Fig. 10. Biomass and catch trajectories obtained in the constant *b* case: for the actual situation, the best allocation scheme, and two levels of reduction of total effort: 95% (reduction 1) and 72% (reduction 2). Reduction 1 yields a growth rate close to that obtained from the effort allocation that maximizes both population and catch levels, whereas reduction 2 yields a growth rate of 1.



In addition, only large fish are caught, which may be desirable for commercial reasons. Under the best allocation of fishing effort, both biomass and catch are maximal whatever the assumption about reproduction, but the contrast in catch between the different effort allocations is much less striking in the constant recruitment case (Figs. 5 and 6). Moving from the actual allocation to the best one would result in multiplying total catch by 4 in the constant *b* case, but would only increase it by 21% in the constant *R* case.

In the constant *b* case, catch and biomass levels have to be considered with caution, because they depend on both initial population sizes and projection horizon. So it is interesting to study what would happen in a transient situation and at different population levels. We assumed that the actual situation corresponds to α and β equal to 0.2, and let stock sizes adjust for a few years. We then modified the fishing effort allocation so that it corresponds to the best scheme ($\alpha = 1$ and $\beta = 0$). This change was introduced in different years to evaluate the efficiency of the best scheme at several population levels (Fig. 9). The best allocation scheme always allows the population to rebuild because the population growth rate does not depend upon initial population sizes. More interesting are catch trajectories (Fig. 9). Moving from the actual effort allocation to the best effort allocation induces a short-term loss in catch. When this change occurs early enough, catch loss is limited to ca. 15%, but if it takes place when the population level is already very low, it may be higher. In general, the short-term loss in catch is found to affect the first 2 or 4 years following the change. This may be compared with short-term losses resulting from total effort reductions (see next section).

Evaluation of spatio-temporal management measures

By accounting for seasons and patches, our model explicitly describes the seasonal segregation of successive population stages in relation to the population life cycle. This enables us to contemplate a wider range of management measures, including openings and closures of fishing zones and (or) fishing seasons. Such spatio-temporal measures may be compared with a global measure like total effort limitation, for instance.

Consider the best allocation scheme found in the analysis, i.e., fishing exclusively on spawner concentrations. In these conditions, the population growth rate is around 1.05. To attain this growth rate under the current allocation scheme, it would be necessary to decrease total effort by 95%, whereas under the best allocation scheme, total effort would remain unchanged. If we were only aiming at a growth rate of 1, a 72% reduction of total effort would still be required. Biomass trajectories exemplify the differences in growth rates (Fig. 10). In fact, the best allocation scheme is very conservative for the stock. Under this scheme, total effort may even be substantially increased without decreasing the population growth rate below 1.

Regarding catch, the advantage of reallocating effort is even more striking. First, although population growth rates are similar, catch is always much lower under the global measure than under the best allocation scheme (Fig. 10). In addition, global measures induce short-term losses in catches (with respect to the status quo) that last longer (7 or 8 years) and are more severe than those implied by reallocating effort (bottom of Fig. 10). Recall that the magnitude of short-term loss is to

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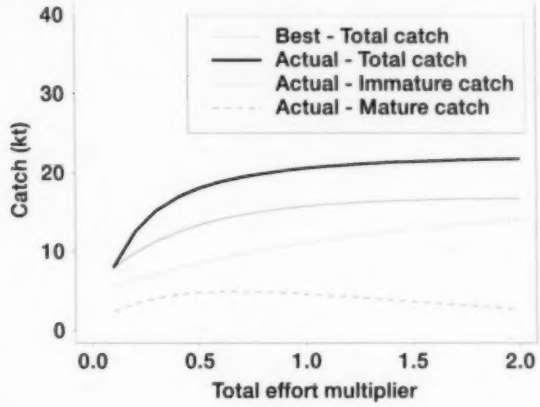
Fig. 11. Equilibrium catch (constant R case) as a function of total effort multiplier (1 corresponding to actual total effort) for two fishing effort allocations: $\alpha = 0$ and $\beta = 1$ (Best), and $\alpha = 0.2$ and $\beta = 0.2$ (Actual).



some extent tied to the initial conditions for the stock (see previous section).

Let us consider this question under the assumption of a constant recruitment R. Then, biomass level is not an issue any longer. Equilibrium catches were computed for a range of effort multipliers under the best and the actual effort allocation (Fig. 11). In the actual situation, the actual equilibrium catch is ca. 14 500 t, as opposed to a maximum of ca. 19 500 t under the best allocation scheme. This catch level cannot be attained by increasing the total effort under the actual effort allocation (the maximal catch for the actual allocation is only around 16 000 t). Therefore, changing effort allocation and total effort enables an increase of 30% in total catch at equilibrium. In this case, short-term losses in catch are minor.

In general, the global measure performs poorly with respect to catch because the exploitation pattern remains the same and there is no change in the age composition of catches. In contrast, when moving to the best allocation scheme, only mature fish are caught and immature fish are no longer exploited. Therefore, the short-term loss in total catch is moderate. Avoiding or mitigating short-term losses in catch is a desirable property for any management measure that aims at reducing overexploitation. Such transitional phases have for instance been studied by Beverton and Holt (1957) and Pelletier and Laurec (1992).

Conclusions

With this model, it is thus possible to show how effective some spatio-temporal management measures may theoretically be. They entail simultaneously a better state of the population and higher catches, when compared with a global measure like total effort limitation. These conclusions have been made possible because this model explicitly accounts for the dependence of exploitation features upon the population life cycle. The main feature, the concentration of fishing on spawners during winter, is captured by a rather simple two-patch, two-season model with migration, which suffices to depict the essentials of the fishery dynamics. Congregation of spawners for reproduction is a frequent feature of the life cycle of many demersal species. What may vary is the extent of the spawning area with respect to the total distribution area. The concentration of exploitation at a place or a moment where fish are more vulnerable because they are grouped also occurs in other instances. In these situations, it is thus important to assess the stock from a model that explicitly accounts for these relationships between exploitation and population. When there is a diagnostic of overexploitation, it may sometimes be possible to address this problem at least partially by resorting to spatio-temporal management measures like opening and closing sensitive zones during a given period of the year. In this paper, we did not evaluate the technical and political feasibility of such measures, which is beyond our competence.

Lastly, the above analysis may be improved in two respects. First, the evaluation of the effort allocation prevailing in the 1980s would be more accurate if data for the inshore fleet had been included. This would require a standardization of fishing effort and catchabilities between the industrial and the inshore fleet, which would probably prove difficult to achieve. Second, some biological parameters are only crude estimates, like the effective fecundity coefficient, recruitment coefficient, and natural mortality coefficient. Note incidentally the insensitivity of our results to the migration rate. Although this depends to some extent on certain characteristics of the system studied, this insensitivity is noteworthy because the difficulty of estimating migration rates is sometimes presented as an impediment to developing spatial models.

The spatio-temporal model presented includes several strong assumptions (particularly about reproduction), the consequences of which were evaluated in the previous section. Among these assumptions, this model is a static description of the system, in that biological parameters and survival rates are constant over time and independent of population sizes. In the constant recruitment case, this leads to an equilibrium situation after a generation time. But in the constant b case, the possible population dynamics emphasize that this model should be used only for diagnostic purposes over a limited time horizon. It is not appropriate for predicting population and catch dynamics, nor for exploring long-term diagnostics. In this analysis, we use it to compare fishing effort allocations, mostly over a generation time. Besides, it is likely that catchabilities, effective fecundity coefficients, and recruitment are affected by density-dependent effects when population levels are drastically modified. In this respect, the strongly contrasting biomasses and catches obtained under extreme effort allocations are probably too optimistic. Unfortunately, no information about possible density-dependent effects is available because of the lack of contrast in exploitation features over years. Another shortcoming of this static description is that this model cannot capture possible effort dynamics with respect to the abundance of the target population, unlike predator–prey type models (Allen and McGlade 1986). This is illustrated by the change in fishing strategies observed in the data after 1991, when fishermen started to target deep species in addition to saithe. It is likely that in most cases, fishing strategies will depend upon the abundances of several species and not only one. Therefore, any model that accounts for effort dynamics should include multispecific considerations. This seems to be a major avenue for future fisheries modelling.

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Acknowledgements

We thank Carl Walters, Jeremy Collie, and Alejandro Anganuzzi for substantially improving the paper. We also thank Alain Biseau for formatting and providing the logbook data.

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Appendix

Construction of the spatio-temporal model

Consider the two-patch, two-season dynamics described in the second section. Let $N^{AB}(t)$ be the vector of population sizes in each patch at time t:

$${}^{t}N^{AB}(t) = \left(N_0^A(t), N_1^A(t),\ldots,N_{n-1}^A(t), N_0^B(t), N_1^B(t),\ldots,N_{n-1}^B(t)\right)$$

where n is the number of age groups (including 0 group) and ${}^{t}N^{AB}(t)$ denotes the transposed vector of $N^{AB}(t)$.

Under the assumption of a constant recruitment rate at age 1, the evolution of $N^{AB}(t)$ may be described via a Leslie matrix L:

$$(A.1)\quad N^{AB}(t+1) = LN^{AB}(t)$$

In general, L can be written as:

$$L = \begin{pmatrix} L_A & Q_{BA} \\ Q_{AB} & L_B \end{pmatrix}$$

where the block matrices L_A, L_B, Q_{AB}, and Q_{BA} correspond to the survival and movement of the individuals present in patch A who survive and stay in patch A, those present in patch B who survive and stay in patch B, those present in patch A who survive and migrate to patch B, and those present in patch B who survive and migrate to patch A, respectively.

To construct the population matrix L for the entire year, we consider each season separately. To avoid a tedious presentation of every intermediate matrix, we only express population sizes in each patch at the end of each season, i.e., $N^{AB}(t + l_i)$ and $N^{AB}(t + 1)$ where l_i denotes the length of season i.

First season (January to April)

By convention, the fish change age groups right after time t at the beginning of the year. Reproduction takes place before fishing at the beginning of this season, so that the number of young-of-the-year at the end of this season is given by:

$$N_0^A(t + l_1) = \sum_{a=5}^{10} b_a^* N_{a-1}^A(t)$$

where b_a^* is the effective fertility coefficient at the end of the first season for individuals of the ath age group, so that b_a^* accounts for the natural mortality of the juveniles during that season. The lag in indices between b_a^* and N_{a-1}^A in the previous equation is due to the convention for changing age groups. Individuals of age 4 at time t are 5 years old and therefore spawn at time $t + l_1$.

The other immature age groups are confined to and exploited in patch B:

$$N_a^B(t + l_1) = N_{a-1}^B(t) \exp\left(-(F_1^B(a,t) + M)\, l_1\right), \qquad \forall a = 1,\ldots,4$$

Mature fish are first confined to and exploited in patch A, and then some of them migrate instantaneously at the end of the season (*i*) toward patch B (*m*) and (*ii*) northeastwards (*e*), so that

$$\begin{cases} N_a^A(t + l_1) = N_{a-1}^A(t)(1 - m - e) \\ \qquad\qquad \exp\left(-(F_1^A(a,t) + M)\, l_1\right) \\ N_a^B(t + l_1) = N_{a-1}^A(t)\, m \exp\left(-(F_1^A(a,t) + M)\, l_1\right), \\ \qquad\qquad \forall a = 5,\ldots,10 \end{cases}$$

Note that $m + e$ has to be smaller than 1.

Second season (May to December)

During this season, the number of young-of-the-year only decreases as a result of natural mortality. They migrate toward the coastal patch (patch B) at the end of the year, so that

$$N_0^B(t + 1) = p_0 N_0^A(t + l_1)$$

where p_0 is the survival rate of the young-of-the-year during the second season. With the exception of age group 4, the other immature fish remain in patch B:

$$N_a^B(t + 1) = N_a^B(t + l_1) \exp\left(-(F_2^B(a,t) + M)\, l_2\right), \qquad \forall a = 1,\ldots,3$$

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At the end of the season, all future spawners migrate instantaneously toward patch A:

$$\begin{cases} N_4^A(t+1) = N_4^B(t+l_1)\exp(-(F_2^B(4,t)+M)\,l_2) \\ N_a^A(t+1) = N_a^A(t+l_1)\exp(-(F_2^A(a,t)+M)\,l_2) \\ \qquad\qquad + N_a^B(t+l_1)\exp(-(F_2^B(a,t)+M)\,l_2), \\ \qquad\qquad\qquad\qquad \forall a = 5,\ldots,10 \end{cases}$$

Annual dynamics of the population

The population is counted at time t, i.e., the beginning of the year, when immature fish and fish that will spawn are segregated in patches B and A, respectively. Thus, the population at time t may be entirely described by the following vector:

$$N(t) = \begin{bmatrix} N_0^B(t) \\ N_1^B(t) \\ \vdots \\ N_3^B(t) \\ N_4^A(t) \\ \vdots \\ N_{10}^A(t) \end{bmatrix}$$

Then, eq. A.1 simplifies to

$$N(t+1) = LN(t)$$

where L is the following Leslie matrix:

$$L = \begin{pmatrix} 0 & 0 & 0 & 0 & b_5 & \cdots & b_{10} \\ p_1 & 0 & 0 & \cdots & \cdots & \cdots & 0 \\ 0 & p_2 & 0 & & & & \vdots \\ 0 & 0 & \ddots & \ddots & & & \vdots \\ \vdots & & \ddots & \ddots & \ddots & & \vdots \\ 0 & & & 0 & \ddots & 0 & 0 \\ 0 & 0 & \cdots & 0 & 0 & p_{10} & 0 \end{pmatrix}$$

and

$$\text{(A.2)} \quad \begin{cases} b_a = p_0 \cdot b_a^*, \quad \forall a = 5,\ldots,10 \\ p_a = \exp(-(F_1^B(a,t)\,l_1 + F_2^B(a,t)\,l_2 + M)), \\ \qquad\qquad\qquad\qquad \forall a = 1,\ldots,4 \\ p_a = (1-m-e)\exp(-(F_1^A(a,t)\,l_1 + F_2^A(a,t)\,l_2 + M)) \\ \quad + m\exp(-(F_1^A(a,t)\,l_1 + F_2^B(a,t)\,l_2 + M)), \\ \qquad\qquad\qquad\qquad \forall a = 5,\ldots,10 \end{cases}$$

Note that b_a is the effective fecundity coefficient after 1 year. The model can accommodate alternative maturity curves by changing the first row of the matrix appropriately.

In the case where recruitment is constant, the population dynamics cannot be framed into a Leslie matrix; survival rates remain unchanged (cf. eq. A.2) but the number of young-of-the-year is simply given by:

$$N_0^B(t+1) = R$$

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OBITUARY/NÉCROLOGIE

Raymond John Heaphy Beverton, CBE, FRS 1922–1995

After a short illness, Ray Beverton died on 23 July 1995, aged 72. He is survived by his wife, Kathy, and three daughters. The sad event of his passing is mourned by fisheries scientists throughout the world. When the founder of a new way of thinking — a paradigm — dies, we are caused to reflect on our science.

What can I know? What ought I to do? What may I hope for? Immanuel Kant (1781) said that these are the only three questions asked of the world by humans: all others are variants on the theme. Kant averred that if a religion or philosophy can provide consistent and satisfactory answers to these questions, it will flourish. By this criterion, Ray Beverton's work with Sidney Holt in devising the catch equation just after the Second World War has provided answers to these questions that remain embedded in almost all fisheries research for the past 40 years. Why else would anyone reissue in facsimile a text published in 1957 in 1993? (Beverton and Holt 1957).

To answer our philosopher: what we can know is the dynamics of fish stock structure; what we ought to do is gather age-structured data partitioned by space and time; what we may hope for is sustainable fisheries. Beverton and Holt's work formed the credo of what Peter Larkin has called the stained glass cathedral period of fisheries science. Although we are now well aware that the credo has been found not sufficient on its own for the sustainable management of our fisheries, the approach developed by Beverton and Holt is now a necessary part of fisheries models and assessment.

Although earlier work by Baranov, Ricker, Thompson, and Bell addressed the same problem, Beverton and Holt provided a stable and practical solution that was quickly adopted worldwide. The first version of the catch integral was worked out by H.R. Hulme, a mathematician, while plotting shell trajectories on top of a tank turret during the Normandy landings, and he became Beverton and Holt's first coauthor in 1947 (Hulme et al. 1947). Michael Graham, then head of the United Kingdom's Lowestoft Fisheries laboratory, and part of the movement that was bringing mathematics into biology, encouraged them, in what Holt has described as a "period of extraordinary harmony" (Holt 1995), to produce a practical integral by adopting Ludwig von Bertalanffy's new theory of asymptotic growth. This was the key that made the thing work and the foundation of the magnum opus, eventually published after a series of papers, as a book (known as the Bible) in 1957. The many integral symbols in the book were unfamiliar to fishery scientists at the time: Benny Schaefer admired Beverton and Holt's 'book of flute music.'

Like many important events that shape the future, it all nearly didn't happen. On his new powerful motorbike, Beverton was whisking Holt back to Cambridge when they crashed into a delivery van, landing Ray in hospital for awhile. Moreover, both Beverton and Holt suffered from seasickness, so much so that Ray Beverton drafted, but did not mail, letters of resignation on his first cruise to the Arctic. Later he fought off nausea by playing canasta on research cruises.

The two pioneers went their separate ways in the 1960s: Sidney Holt moved to FAO in Rome and later worked for the International Whaling Commission. Ray Beverton became, for 15 years, a very effective first Head of the Natural Environment Research Council (NERC) in the United Kingdom, establishing the ground rules for the new Council and overseeing a move from London, where NERC occupied rather seedy offices in the Charing Cross Road, to Swindon, then a sleepy provincial market town. One legacy of Ray's success as a leader of decentralised administration that he doubtless did not expect was the establishment of a good railway service between London and Swindon. (Canadian readers will no doubt have guessed that the top UK government bureaucrats did NOT move to the country from London.) Returning to an academic career in the early 1980s with scarcely a hiccup, Ray Beverton helped oversee a merger at the University in Cardiff, became a well-liked head of department, promoted and taught a most successful master's course in fisheries, and was elected as a Fellow of the Royal Society.

The term 'committee man' is used pejoratively by frustrated academics around the globe, but Ray Beverton was a tireless and effective committee member in the best and most productive sense of the term. He had developed the knack of manoeuvring committees to do good and sensible things rather than fighting amongst each other, which is arguably the job they are most suited for. For example, I saw him in action as President of the Fisheries Society of the British Isles (FSBI) in the mid 1980s where he persuaded that previously ultracautious society to spend its considerable journal royalties on student scholarships, and later, on student travel grants. He good-naturedly convinced the Society's council that his policy was a 'tradition' (when it patently wasn't), and even today the Society, which now awards an annual Beverton Medal, is obliged to do this good work. Ray's legacy, therefore, has been to lend cohorts of graduate students a helping hand at a critical phase of their careers. Moreover, few of us who were meeting him regularly at two or three venues or conferences each year appreciated just how diverse Ray's work became at that time. He was continuously travelling and injecting common sense into fisheries panels from Lake Malawi, the Black Sea, and Chile to the fjords of Bergen. He was editing the Journal du Conseil for ICES and working for UNESCO, the World Wildlife Fund, the Intergovernmental Oceanographic Commission, and the UK Challenger Society while at the same time deliv-

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ering a stimulating series of keynote addresses to international symposia (e.g., Beverton 1989, 1990, 1992, 1994).

As if it were not enough to found a new science, Ray Beverton published new and stimulating papers to the end. Their principal theme was how life history characters determine sustainability. Most readers of *The Canadian Journal of Fisheries and Aquatic Sciences* will not dispute that the current state of world fish stocks is deeply depressing. Ray was well aware of this problem: in the paper he was drafting when he fell ill he states that "this is not what Sidney and I were hoping for when we embarked on our immediate post-war endeavours in those heady days of the late 1940s." I am delighted to report that Kathy Beverton, who is to collect the first FSBI Beverton Medal on Ray's behalf in July 1996, has found detailed speaking notes and figures for Ray's final paper, which was to have been the first Larkin Lecture at the Fisheries Centre at the University of British Columbia. In 1997, an edited version will be published in a Beverton and Holt Jubilee Issue of *Reviews in Fish Biology and Fisheries*, the journal that Ray, typically, supported as a founder member of the Editorial Board in 1989. Ray was optimistic that fisheries science is in good shape (Beverton 1989) to meet the challenges facing it. It is a testament to Ray Beverton's stature as a fishery scientist that his latest work will be found fresh and useful 50 years after he started.

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ERRATUM

Erratum: Uptake rates of food-chain and waterborne mercury by fish: field measurements, a mechanistic model, and an assessment of uncertainties

John R. Post, Rena Vandenbos, and Donald J. McQueen

Ref: Can. J. Fish. Aquat. Sci. **53**: 395–407 (1996).

In Table 3 (p. 401) the units for methyl mercury concentration in water should read $ng \cdot L^{-1}$ (not $\mu g \cdot g^{-1}$, which is correct, however, for concentration in biota). The Publisher apologizes for this unfortunate typesetting error.

Received at NRC June 28, 1996.
J11896

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Contents (concluded) / Table des matières (suite et fin)

OBITUARY — NÉCROLOGIE

1200–1201 — Raymond John Heaphy Beverton, CBE, FRS

ERRATUM — ERRATUM

John R. Post, Rena Vandenbos, and Donald J. McQueen — **1202** — Erratum: Uptake rates of food-chain and waterborne mercury by fish: field measurements, a mechanistic model, and an assessment of uncertainties

Marlene S. Evans, Michael T. Arts, and Richard D. Robarts	**1048–1060**	Algal productivity, algal biomass, and zooplankton biomass in a phosphorus-rich, saline lake: deviations from regression model predictions
David R. Montgomery, John M. Buffington, N. Phil Peterson, David Schuett-Hames, and Thomas P. Quinn	**1061–1070**	Stream-bed scour, egg burial depths, and the influence of salmonid spawning on bed surface mobility and embryo survival
Kenneth A. Rose, Jeffrey A. Tyler, R. Christopher Chambers, Grace Klein-MacPhee, and Donald J. Danila	**1071–1091**	Simulating winter flounder population dynamics using coupled individual-based young-of-the-year and age-structured adult models
E.S. Millard, D.D. Myles, O.E. Johannsson, and K.M. Ralph	**1092–1111**	Phytoplankton photosynthesis at two index stations in Lake Ontario 1987–1992: assessment of the long-term response to phosphorus control
E.S. Millard, D.D. Myles, O.E. Johannsson, and K.M. Ralph	**1112–1124**	Seasonal phosphorus deficiency of Lake Ontario phytoplankton at two index stations: light versus phosphorus limitation of growth
S. Perin, F.R. Pick, D.R.S. Lean, and A. Mazumder	**1125–1132**	Effects of planktivorous fish and nutrient additions on primary production of shallow versus deep (stratified) lake enclosures
Isabelle Larocque, A. Mazumder, M. Proulx, D.R.S. Lean, and F.R. Pick	**1133–1142**	Sedimentation of algae: relationships with biomass and size distribution
David L. Strayer, Jon Powell, Peter Ambrose, Lane C. Smith, Michael L. Pace, and David T. Fischer	**1143–1149**	Arrival, spread, and early dynamics of a zebra mussel (*Dreissena polymorpha*) population in the Hudson River estuary
Réjean Fortin, Pierre Dumont, and Sylvie Guénette	**1150–1156**	Determinants of growth and body condition of lake sturgeon (*Acipenser fulvescens*)
Stephen C. Weeks	**1157–1164**	A reevaluation of the Red Queen model for the maintenance of sex in a clonal–sexual fish complex (Poeciliidae: *Poeciliopsis*)
Sheldon J. McKay, Robert H. Devlin, and Michael J. Smith	**1165–1176**	Phylogeny of Pacific salmon and trout based on growth hormone type-2 and mitochondrial NADH dehydrogenase subunit 3 DNA sequences
J.H. Clemons, L.E.J. Lee, C.R. Myers, D.G. Dixon, and N.C. Bols	**1177–1185**	Cytochrome P4501A1 induction by polychlorinated biphenyls (PCBs) in liver cell lines from rat and trout and the derivation of toxic equivalency factors
Dominique Pelletier and Pierre Magal	**1186–1199**	Dynamics of a migratory population under different fishing effort allocation schemes in time and space

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Canadian Journal of Fisheries and Aquatic Sciences

Volume 53, Number 5, May 1996

Journal canadien des sciences halieutiques et aquatiques

Volume 53, numéro 5, mai 1996

ARTICLES		ARTICLES
Jeffrey A. Hutchings	**943–962**	Spatial and temporal variation in the density of northern cod and a review of hypotheses for the stock's collapse
Marianne Vignaux	**963–973**	Analysis of spatial structure in fish distribution using commercial catch and effort data from the New Zealand hoki fishery
Michael D. Tringali and Theresa M. Bert	**974–984**	The genetic stock structure of common snook (*Centropomus undecimalis*)
Atle Hindar, Frode Kroglund, Espen Lydersen, Anja Skiple, and Rolf Høgberget	**985–993**	Liming of wetlands in the acidified Lake Røynelandsvatn catchment in southern Norway: effects on stream water chemistry
Hélène Cyr and Robert H. Peters	**994–1006**	Biomass-size spectra and the prediction of fish biomass in lakes
Holger Hovgård	**1007–1013**	A two-step approach to estimating selectivity and fishing power of research gill nets used in Greenland waters
Holger Hovgård	**1014–1017**	Effect of twine diameter on fishing power of experimental gill nets used in Greenland waters
Peter D. Premdas and Chris D. Metcalfe	**1018–1029**	Experimental transmission of epidermal lip papillomas in white sucker, *Catostomus commersoni*
Selina M. Stead, Dominic F. Houlihan, H. Anne McLay, and Ray Johnstone	**1030–1037**	Effect of ration and seawater transfer on food consumption and growth of Atlantic salmon (*Salmo salar*) smolts
Renato Vitic and Curtis Strobeck	**1038–1047**	Mitochondrial DNA analysis of populations of lake trout (*Salvelinus namaycush)* in west-central Canada: implications for stock identification

Continued on inside back cover / Suite au verso

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Printed in Canada / Imprimé au Canada par
University of Toronto Press Inc.
Typesetting by / Typographie par
NRC Research Press / Presses scientifiques du CNRC

0706-652X(199605)53:5;1-4